Control of Biofouling on Reverse Osmosis membranes using DBNPA

by

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DECLARATION

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ABSTRACT

Reverse Osmosis (RO) is used throughout the world for water desalination and it has gained wide popularity due to its efficient energy consumption and the safe operating process. Fouling (of which biological fouling is the most problematic) of the membranes is, however, an inevitable process that cannot be avoided, only managed. Biological fouling is the growth of microorganisms in the membrane system, causing undesirable effects. The correct pre-treatment can reduce (but not necessarily prevent) biofouling. This is because microorganisms have the ability to reproduce and form secondary populations throughout the membrane system, even if 99.99% of the microorganisms are removed in the pre-treatment process.

Most modern RO plants are equipped with thin film composite polyamide (TFC PA) membranes. However, biological control on such membranes is restricted, since oxidising biocides like chlorine degrade the membrane material, thereby increasing salt passage and reducing membrane life. Therefore, this study investigated the use of a common non-oxidising biocide, *i.e.* 2,2-dibromo-3-propionamide (DBNPA) to manage biological growth on TFC PA membranes.

The primary aim was to demonstrate the influence of three DBNPA dosing variables on the control of biofouling on the RO membranes. These variables were dosage (10 ppm to 200 ppm), dosing frequency (twice daily to once every 2nd day) and dosing duration (30 min to 2 hours). The work also strongly relied on the characterisation of biological fouling through determination of biomass parameters (protein concentration, polysaccharide concentration, total cell count and colony-forming units) and linking it to flux decline.

Tests were conducted in lab-scale RO membrane blocks, housing flat-sheet TFC PA membranes with appropriate flow spacers typically found in commercial spiral-wound membrane cartridges. Since clean municipal water was used as feed water, nutrients (sodium acetate, sodium nitrate and sodium dihydrogen orthophosphate, in the ratio of 100:20:10 to give a final carbon concentration of 100 µg/ℓ) were supplemented to stimulate sufficient microbial growth, thereby enabling a sensible study on the effect of DBNPA dosing.

During the removal of the biofilm from the membrane, no combination of the removal and homogenisation techniques (e.g. scraping the biofilm from the membrane, ultrasonic bath and ultrasonic probe treatment) yielded significantly higher colony forming unit (CFU) counts. R_2A agar, however, produced significantly higher CFU counts compared to nutrient agar. Therefore, the agar used during plate counts appears to have been of greater significance on cell enumeration than the combination of biofilm removal and homogenisation techniques, which had little effect on cell counts, irrespective of agar used.

DBNPA dosing reduced the amount of biofouling, regardless of the dosing strategy used. However, within the scope of this study, biofouling was best controlled with a DBNPA dosage of 100 ppm for two hours once per day. Applying the same dosing strategy every second day, was not as effective in limiting flux decline, but still produced better results than the remaining dosing strategies. This supports the notion of a sufficiently high dosage for an optimal time, rather than high concentration shock-dosages for a short period.

A significant increase in biomass parameters (cell count, colony forming units, and protein- and polysaccharide concentration) was observed when nutrients were added to the feed water. Protein concentration ($p=4.29 \times 10^{-5}$, $R^2=0.71$) and polysaccharide concentrations ($p=0.0053$, $R^2=0.58$) on the membrane had a strong and significant relationship with the flux decline, making it suitable parameters for biofouling quantification. CFU showed a significant, but not strong, ($p=0.0011$, $R^2=0.54$) relationship to the flux decline, whereas total cell count did not provide a significant ($p=0.14$) relationship. Protein- and polysaccharide concentrations could therefore be used for the quantification of the biofouling. A destructive study should, however, be performed to determine these parameters. A practical tool is therefore still necessary for the early diagnosis of biofouling.

For future studies, it is recommended that larger ranges of cross-flow velocities and pressures be investigated, together with the effect of DBNPA dosing. Ideally, the work should be performed on a membrane that is packed in a spiral-wound format to simulate real-life situations.

OPSOMMING

Tru-osmose word wêreldwyd vir waterontsouting gebruik en is weens effektiewe energie gebruik en veilige bedryfsproses baie populêr. Bevuiling (waarvan biologiese bevuiling die problematiesste is) van die membrane is ‘n onvermydelike proses wat slegs bestuur kan word. Biologiese bevuiling onstaan weens mikrobiese groei wat binne die membraansisteem plaasvind en sodoende verskeie ongewenste probleme tot gevolg het. Doeltreffende voorbehandeling kan biologiese bevuiling verminder, maar nie noodwendig verhoed nie. Dit is a.g.v. mikro-organismis se vermoë om voort te plant en sekondêre kolonies regdeur die membraanstelsel te vorm, selfs as 99.99% van die organismes tydens voorbehandeling verwyder.

Die meeste moderne tru-osmosis aanlegte is met dun film saamgestelde poliamied membrane toegerus. Biologiese beheer op die membrane is beperk aangesien oksiderende biododers, soos chloor, die membraan degradeer. Dit veroorsaak dan dat die soutdeurlating verhoog en membraanleeftyd afneem. Om hierdie rede word die gebruik van ‘n nie-oksiderende biododer, naamlik 2,2-dibroom-3-propionamide (DBNPA), op dun film saamgestelde poliamied membrane in hierdie studie ondersoek.

Die primêre doel van die studie was om die invloed van drie DBNPA doseringsveranderlikes op die beheer van biobevuiling of tru-osmose membrane te demonstreer. Die veranderlikes was dosering (100 dpm tot 200 dpm), doserings intervalle (twee keer per dag tot elke tweede dag) en doseringstyd (30 min tot 2 ure). Die werk steun ook op die karakterisering van die biologiese bevuiling deur van biomassa parameters (proteïen- en polisakkariedkonsentrasies, totale sel telling en kolonievormende eenhede) gebruik te maak wat aan die afname in stroming gekoppel kan word.

Toetse is in laboratoriumskaal, tru-osmose membraanblokke uitgevoer wat in staat was om dun film saamgestelde poliamied membrane saam met die toepaslike voerspasieerders te huisves, soos tipies in industriële stelsels aangetref word. Aangesien skoon munisipale water vir voerwater gebruik is, is voedingstowwe (natriumasetaat, natriumnitraat en natrium diwaterstofortofosfaat, in die verhouding van 100:20:10, gebruik om ‘n finale koolstofkonsentrasie van 100 µg/ℓ te gee) by die voerwater gevoeg om voldoende mikrobiese groei te stimuleer en sodoende ‘n sinvolle studie van die effek van DBNPA dosering uit te voer.

Gedurende die verwydering van die biofilm vanaf die membraan, is gevind dat geen verwyderings- en homogeniseringstegniek (b.v. skraping van die biofilm vanaf die membraan, ultrasoniesebad en ultrasoniesestang behandeling) beduidend meer kolonievormende eenhede opgelewer het nie. R_2A agar het wel beduidend meer kolonievormende eenhede opgelewer in vergelyking met voedingsagar.

Die agar wat gebruik word is dus meer beduidend as die biofilm verwyderings- en homogeniseringstegniek wat gebruik word.

DBNPA dosering het die hoeveelheid biobevuiling verminder, ongeag die doseringstrategie wat gebruik was. Binne die omvang van die studie, is biobevuiling die beste beheer deur ‘n DBNPA konsentrasie van 100 dpm vir twee ure elke dag. Deur dieselfde doseringsstrategie elke tweede dag toe te pas, was minder doeltreffend om die stromingsafname teen te werk. Dit was egter meer effektief as die ander doseringstrategieë wat getoets is. Die resultate ondersteun dus die idee van ‘n voldoende hoë doseringskonsentrasie vir ‘n optimale tyd, eerder as hoë doseringskonsentrasies op ‘n kort skok basis.

‘n Beduidende toename in biomassa parameters (totale seltelling, kolonievormende eenhede, proteien- en polisakkariedkonsentrasies) is waargeneem wanneer voedingstowwe by die voerwater gevoeg was. Proteïenkonsentrasies ($p=4.29 \times 10^{-5}$, $R^2=0.71$) en polisakkariedkonsentrasies ($p=0.0053$, $R^2=0.58$) het ‘n sterk en beduidende verwantskap met die afname in stroming gehad. Dit maak die twee parameters geskik om vir die kwantifisering van biobevuiling gebruik te word. Kolonievormende eenhede het ook ‘n beduidende, maar minder sterk, ($p=0.0011$, $R^2=0.54$) verwantskap met stromingsafname gehad. Totale seltelling het egter geen beduidende ($p=0.14$) verwantskap getoon nie. Proteïen- en polisakkariedkonsentrasies kan dus gebruik word vir kwantifisering van biobevuiling terwyl kolonievormende eenhede minder geskik is en totale sel telling glad nie geskik is nie. ‘n Destruktiewe studie is egter nodig om die parameters te bepaal. ‘n Praktiese manier is dus nog nodig om vroeë biobevuiling te identifiseer.

Daar word aanbeveel dat ‘n groter verskeidenheid kruisvloei snelhede en drukke ondersoek moet word tesame met die effek van DBNPA dosering vir toekomstige studies. Dit sal ook meer gewens wees as die studies op spiraalgewikkelde membrane gedoen word om werklike prosesse beter te simuleer.

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NOMENCLATURE

Symbol	Description	Units
ΔP_a	Actual differential pressure	kPa
ATP	Adenosine Triphosphate	pg/cm^2
A	Area	m^2
AAP	Average trans-membrane pressure	kPa
C_c	Concentrate concentration	mg/ℓ
$\Delta\pi$	Differential osmotic pressure	kPa
X_i	Dissolved specie concentration	kg.mol/m^3
CF	Feed concentration	mg/ℓ
Q_F	Feed flow rate	m^3/h
P_{feed}	Inlet pressure	kPa
l	Length	m
Q_{JW}	Membrane flux	LMH (ℓ /m^2.h)
π	Osmotic pressure	kPa
Q_P	Permeate flow rate	m^3/h
ppm	Parts per million	mg/ℓ
$P_{permeate}$	Permeate pressure	kPa
ΔP	Pressure difference	kPa
R	Recovery	%
C_{FB}	Salt concentrations at boundary layer	mg/ℓ
C_P	Salt concentrations in permeate	mg/ℓ
C_F	Salt feed concentration	mg/ℓ
C_C	Salt concentration of concentrate	mg/ℓ
Q_{JS}	Salt flow rate through membrane	kg/s
Ks	Salt permeability coefficient	m^3/(m^2.s)
T	Temperature	K
t	Time	s
R	Universal gas constant	kJ/(kg.K)
K_w	Water permeability coefficient	m^3/(m^2.s.kPa)

Abbreviations	
Colony-forming units	CFU
% Rejection	R
2,2-dibromo-3-propionamide	DBNPA
Average permeate flow divided by number of membrane modules	EPF
Calcium carbonate precipitation potential	CCPP
Clean in place	CIP
Concentrate flow	CF
Concentration factor	Z
Concentration of feed-concentrate	CFC
Deionised water	DI

Granular-activated carbon	GAC
Extracellular polymeric substances	EPS
Permeate flow	PF
Salt passage	%SP
Salt transport temperature correction factor	STCF
Temperature correlation factor	TCF
Thin-film composite polyamide	TFC PA
Total organic carbon	TOC
Subscripts	
Su	Start-up
A	Actual condition
S	Standard condition
s	Salt
c	Concentrate
p	Permeate
w	Water

GLOSSARY

Term	Description
Biological/biofouling	Fouling caused on the membrane surface and in the feed channel due to excessive biological growth.
Colony forming unit (CFU)	The number of microorganisms present in a sample that is culturable on laboratory culture media.
Concentrate/brine	The stream that did not migrate through the membrane and has a higher concentration of dissolved solids than the feed stream.
Concentration factor	The factor by which the concentration of the dissolved salt in the feed increases.
Concentration polarisation	Concentration gradient that forms from the membrane surface to the bulk fluid.
Cross flow velocity	The velocity of the water that flows across the membrane during operation.
Data normalisation	Data normalisation is used to compare membrane performance at different times by eliminating the influence of temperature, pressure and concentration on the performance.
Extracellular polymeric substances (EPS)	Substances secreted by microorganisms during growth.
Feed channel	Channel along which the water flows over the membrane element.
Feed channel pressure drop	The pressure drop experienced by the water when it flows through the feed channel.

Flux	The rate at which the water migrates through the membrane per membrane area.
Flux decline	The decrease in flux, which can be caused by a variety of factors.
Fouling	Accumulation of unwanted materials on the membrane surface and feed channel causing a decline in performance.
Loopful	In microbiology, the amount of liquid which can be held within the loop of platinum wire used for transferring cultures
Membrane	Selective barrier that can allow only the passage of certain elements while retaining others in a fluid.
Membrane pressure drop	The pressure drop over the membrane from the feed side to the permeate side.
Permeate	The desalinated/pure stream.
Pretreatment	Pretreatment methods used to remove foulants from the feed water to the membranes to minimise fouling on the membranes.
Recovery	The percentage of feed water that leaves as permeate.
Rejection	The percentage of a species rejected by the membrane.
Reverse osmosis (RO)	A separation process used to desalinate saline water by applying a pressure higher than the osmotic pressure and allowing the water to flow through a semi-permeable membrane.
Spiral wound	A common type of packing method used to pack RO membranes.

CHAPTER 1: INTRODUCTION

1.1 Background

More than 40% of people around the world are already affected by water scarcity on almost every continent. It is predicted that by 2025, around two thirds of people around the world will live under water-stressed conditions (OECD/FAO 2012). Water desalination (currently dominated by membrane processes) is one of the methods that can be used to increase the supply of fresh water (Reddy & Ghaffour 2007). The increase in population and expansions in industry and agriculture have already caused countries under water stress to make use of desalination to meet their water requirements (Ghaffour et al. 2013). The main limiting factor for membrane processes is fouling (especially biofouling) that occurs on the membrane surface. Around 70% of seawater reverse-osmosis (RO) plants suffer from biological fouling. Paul (1991) reported that during surveys conducted in the United States, 58 out of 70 RO plants experienced fouling problems with biofouling as the most common type. Fouling of membrane surfaces will increase operating cost, reduce performance and reduce membrane lifetime, which in turn may prohibit the use of membrane technologies for water purification (Vrouwenvelder et al. 2011; Paul & Abanmy 1990).

Many different methods are currently used to reduce and prevent the effect of biofouling on membrane surfaces. A single method is not successful and a combination of methods is usually used. The incorrect use of preventative measurements can lead to very high operating cost (Al-Juboori & Yusaf 2012). Although it is difficult to determine the exact cost because it is comprised of many different factors, which include decreased product quality, membrane replacement, cleaning chemical and shortened plant life, the biofouling market is estimated to be worth billions of dollars every year (Flemming et al. 2011). Even if pretreatment removed 99.99% of the microorganisms, the remaining cells still have the ability to grow and reproduce on the membrane surface. (Flemming et al. 1997).

1.2 Motivation for study

Most modern plants make use of polyamide (PA) membranes for RO. Biological control is, however, restricted, since PA membranes are very vulnerable to chlorine attack. Chlorine degrades the membrane, thereby increasing salt passage and reducing membrane life. Non-oxidising biocides can be used to control microbial growth on the surface of polyamide membranes. A wide variety of non-oxidizing biocides are available e.g. DBNPA (2,2-dibromo-3-propionamide), isothiazolone, formaldehyde, glutaraldehyde, quaternary ammonium and SBS (sodium bisulphite) and long-term use of many of these biocides could cause microbial resistance (Baker & Dudley 1998; Kucera 2010). Of these, DBNPA is a popular, fast acting biocide, which easily hydrolyzes in water. However, limited

studies have been carried out to optimise the use of DBNPA on RO membranes to control biofouling. Table 1-1 summarises the dosages, dosing durations and frequencies recommended in literature.

Table 1-1: Recommended dosages, dosing durations and frequencies of DBNPA

Dosage	Dosing duration	Frequency	Reference
100 ppm	30-60 min	Once every two days to once every seven days depending on microbial growth	(Kucera 2010)
1-2 ppm	Continuous	Continuous	
20 ppm	30-min cycles	-	(Dow Chemical Company 2000)
10-30 ppm	30 min - 3 h	Every five days	(Hydranautics Nitto Group Company 2013)
0,5-2 ppm	Continuous	Continuous	
20 ppm	30-60 min	2-3 times a week with option of once a week, depending on performance	(Bertheas et al. 2009)

Considering the rather conflicting nature of these recommendations, additional studies on the use of DBNPA to control biofouling can certainly contribute to a better understanding and guidance on the effect of variables such as dosage, dosing duration and frequency.

There is also no univocal quantification method in existence whereby biofouling can be linked independently to operational problems, making it difficult to quantify biofouling (Vrouwenvelder et al. 2008). Pressure drop over the membrane due to fouling is a good indicator, but it is not exclusively linked to biofouling, since other types of fouling can also cause a pressure drop. Pressure drop measurements are also not sensitive enough for early detection of bio-growth (Vrouwenvelder et al. 2008). A range of indicators, which include extracellular polymeric substances (EPS) content, cell count and plate count) is available to determine the amount of biofouling on a membrane; however, only a limited amount of studies have been carried out to find a link between these parameters and actual system performance (Vrouwenvelder et al. 2009).

1.3 Aims and objectives

With the motivational discussions in Section 1.2 as background, the primary aim of this study was to provide a better understanding of the effect of three selected operating variables (*i.e.* DBNPA dosage, dosing frequency and dosing duration) on membrane biofouling. As such, an optimal dosing strategy was to be identified within the range limitations of the study. In support of this, the secondary aim was to find a suitable biological parameter that could be linked to system performance, thereby quantifying biofouling better.

This was achieved by pursuing the following objectives:

- Construction of bench-scale experimental equipment that is able to house a membrane sheet, feed spacer and is able to simulate the conditions inside a RO membrane module. This setup should be able to facilitate the growth of a sufficient and diverse microorganism community.
- Test different non-oxidising biocide (DBNPA) dosages, dosing durations and dosing frequencies to determine the best biofouling dosing conditions.
- Evaluate the different biofilm removal and homogenisation techniques (e.g. scraping from the membrane, ultrasonic bath treatment and ultrasonic probe treatment) that are critical in biofilm analysis.
- Finding relationships between typical biomass parameters (cell count, colony forming units, proteins and polysaccharides) and process conditions (flux decline) which can be used for biofilm quantification.

1.4 Limitations of this study

A very wide variety of different microorganisms is responsible for biofouling on reverse-osmosis membranes. The variety is influenced by many factors including, but not limited to, seawater or brackish water desalination, seasonal variations, pretreatment and operating conditions (Zhang et al. 2011; Bereschenko et al. 2010; Khambhaty & Plumb 2011; Ying et al. 2013). Previous studies have used single-, multiple- or naturally occurring bacteria to stimulate growth (Goldman et al. 2009; Suwarno et al. 2012). These methods are limiting, since it is difficult to culture single strains of bacteria on a continuous basis. The bacteria are also not necessarily a good representation of the wide variety of microorganisms encountered on a membrane surface. The naturally occurring bacteria population in water is also not always constant, leading to variations in the biofilm formed.

Biofilms take days to several weeks to form before a notable change in membrane behaviour is observed. Lab studies range from three days up to ten days (Suwarno et al. 2012; Dreszer et al. 2014) with some studies on larger plants spanning several years (Boorsma et al. 2011; Bertheas et al. 2009). Therefore, the number of experiments that could be executed within the practical timeframe of this study was limited.

The study was also limited to flat-sheet membranes, which are much smaller than the spiral wound membrane elements that are commonly used in industry. The hydrodynamics experienced by the biofilm on the flat sheet could therefore be different from the hydrodynamics in spiral wound elements.

CHAPTER 2: LITERATURE REVIEW

2.1 Reverse osmosis overview

The use of reverse osmosis (RO) to desalinate water is not a new concept and is widely used throughout the world (Baker 2004). The desalination process used during RO is based on a membrane-separation method. This is, however, not the only desalination method that is currently available. The other widely used method is thermal desalination (El-Dessouky & Ettouney 2002). The high-energy requirements for thermal desalination made RO a much more desirable method for future applications (Fritzmann et al. 2007). For this reason, this study will focus on RO.

2.1.1 Principle of reverse osmosis

RO is a pressure-driven diffusion process through a membrane, which is used to separate dissolved solids from a solution. Compared to other pressure-driven membrane-filtration systems, RO relies on diffusion for separation, while the other systems rely on size exclusion for rejection. The other systems include microfiltration (MF), ultra-filtration (UF) and nano-filtration (NF). The capabilities of RO compared to the different pressure-driven membrane processes are illustrated in Figure 2-1. MF and UF can be used to remove fine colloidal particles and bacteria. UF can be used to remove viruses and larger molecules such as proteins. Smaller compounds such as dissolved salts can be removed by RO and NF (Fritzmann et al. 2007).

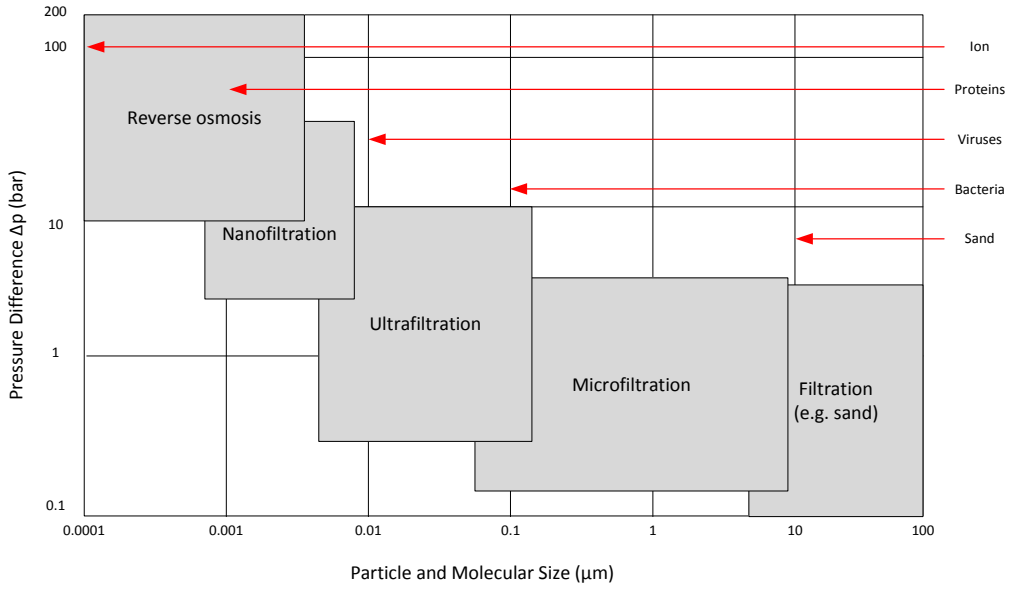


Figure 2-1: The capabilities of pressure-driven processes (redrawn from Fritzmann et al. 2007; Kura 2010)

Reverse osmosis is based on the principle of osmosis, which is the natural flow of a solvent with a low concentration of dissolved solids through a semi-permeable membrane to a solvent containing a high concentration of dissolved solids. Only the solvent is able to pass through the membrane and not the dissolved solids. The process will continue until the concentration of dissolved solids is the same in both compartments (Kucera 2010; Silberberg 2007). This is illustrated in Figure 2-2.

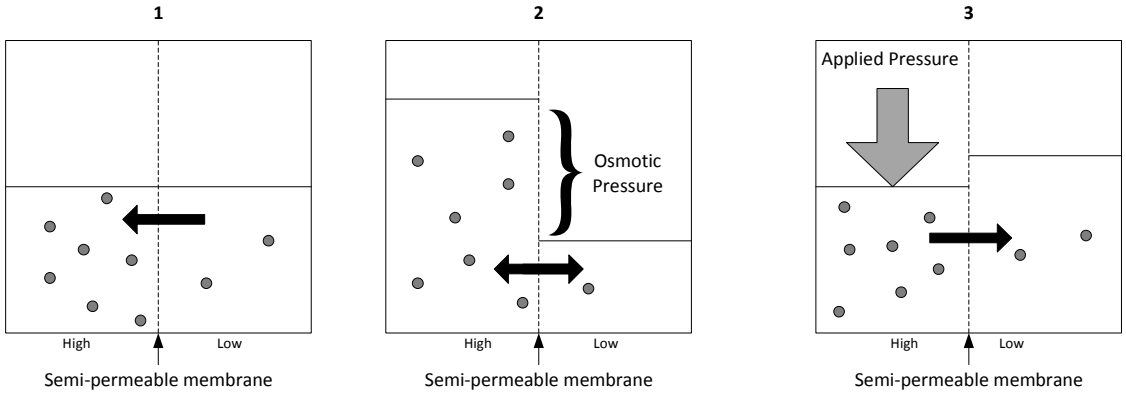


Figure 2-2: Principle of osmosis and reverse osmosis (redrawn from Kucera 2010)

Once equilibrium is reached, a liquid height difference exists between the two compartments. The difference is known as the osmotic pressure (π). The osmotic pressure of a solute is a function of the concentration of dissolved solids (Kucera 2010). The osmotic pressure of a solvent can be determined once the concentration of dissolved species is known.

The osmotic pressure can be calculated as follows (El-Dessouky & Ettouney 2002):

$$\pi = RT \sum X_i \qquad \textbf{Equation 2-1}$$

where

π = Osmotic pressure
R = Universal gas constant
T = Temperature
X_i = Dissolved species concentration

Every 100 ppm of total dissolved solids (TDS) will contribute between 4.1 and 7.6 kPa to the osmotic pressure of the solvent (Kucera 2010).

During reverse osmosis, pressure is applied to the compartment with the high, dissolved solids concentration. The applied pressure is higher than the osmotic pressure and forces the water molecules through the membrane to the compartment containing the low-concentration dissolved solids. The dissolved solids are not able to pass through the membrane, resulting in relatively pure

water (Kucera 2010). The operating pressure required must also be higher than the osmotic pressure to overcome frictional losses, membrane resistance and permeate osmotic pressure (El-Dessouky & Ettouney 2002).

2.1.2 Cross-flow RO unit

Cross flow is used with RO to minimise the fouling and scaling on the membrane surface. During cross-flow filtration, only a certain percentage of the feed water will filtrate/diffuse through the membrane. The rest of the water passes tangentially over the membrane. A permeate (desalinated stream) and a concentrate (containing most of the dissolved solids) effluent streams are then generated (Kucera 2010) as shown in Figure 2-3.

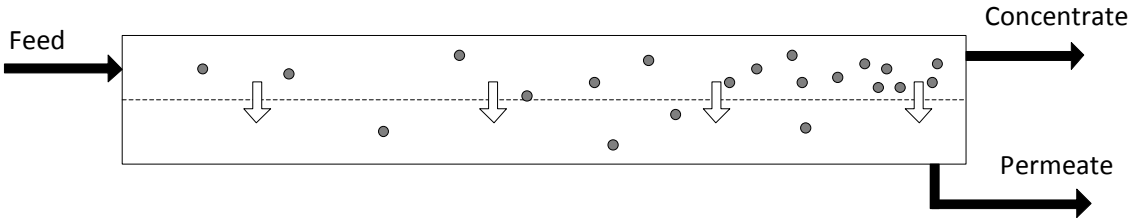


Figure 2-3: Cross-flow filtration where the clear arrows represent the diffusion of water through the membrane

2.1.3 Spiral-wound modules

The most common type of packing for the RO membranes is in the form of spiral-wound modules. A high packing density is achieved through this type of packing. Two membrane sheets with a permeate space between them are placed back to back. Three of the sides are then glued together. The open side is attached to a centre pipe. The permeate is then only able to exit the "leaf" on the one side. Several of these "leafs" are connected to the centre pipe. These "leafs" are then rolled around the centre pipe with a feed spacer between the "leafs" to keep the channel open. The thickness of the mesh can vary to accommodate different feed waters. The feed then enters from the one side of the module and flows over the membrane to facilitate cross flow. The diameter of the spiral-wound module can vary between 0.10 m and 0.20 m with the standard element 0.20 m in diameter. The length can vary between 1.02 m and 1.52 m with the standard length 1.102 m. These modules are then grouped together to form the system. This is discussed in more detail in Section 2.1.11.

2.1.4 Recovery and concentration factor

Recovery is the term used to describe the percentage of feed water that is recovered as permeate. Many brackish desalination systems run at a recovery rate close to 75%, but recoveries can range from 50% to 90% while the recovery rate in seawater desalination plants is between 35% and 45% (Kucera 2010; Baker 2004).

The recovery rate can then be defined as:

$$R = \frac{Q_P}{Q_F} \times 100 \quad \textbf{Equation 2-2}$$

where

R= The % recovered

Q_P= Permeate flow rate

Q_F= Feed flow rate

The concentration factor is the factor by which the concentration of the dissolved salt in the feed increases. The concentration factor can be expressed as:

$$Z = \frac{C_C}{C_F} = \frac{1+(R.J)-R}{1-R} \quad \textbf{Equation 2-3}$$

where

Z= Concentration factor

C_C= Salt concentration of concentrate

C_F= Salt concentration of feed

J= Salt rejection by membrane

The salt rejection is usually very high (>97%). The concentration factor can therefore be estimated as follows:

$$Z = \frac{C_C}{C_F} \approx \frac{1}{1-R} \quad \textbf{Equation 2-4}$$

2.1.5 Flux and driving force

The flow of a liquid through a specific membrane area is defined as the flux through the membrane. In the case of water desalination, the liquid is water. The flux through the membrane is a pressure driven process. An increase in pressure will cause an increase in flux (Kucera 2010).

Water movement through a membrane is given as (El-Dessouky & Ettouney 2002):

$$Q_{JW} = K_w A(\Delta P - \Delta\pi) \quad \textbf{Equation 2-5}$$

where

Q_{JW} = Flux through the membrane

K_w = Water permeability coefficient

A = Membrane area

ΔP = Pressure across membrane

$\Delta \pi$ = Differential osmotic pressure across membrane

The transportation of salts across the membrane is a concentration-driven process and only dependent on the concentration of salts in the feed and permeates streams. Salt transportation through the membrane can be expressed as (El-Dessouky & Ettouney 2002):

$$Q_{JS} = K_S A(C_{FB} - C_P) \quad \textbf{Equation 2-6}$$

where

Q_{JS} = Salt flow rate through membrane

K_s = Salt permeability coefficient

A = Membrane area

C_{FB} = Salt concentrations at boundary layer

C_P = Salt concentrations in permeate

The models presented here are rather basic and only presented to give an idea of the transportation through the membrane (Moonkhum et al. 2010; Malaeb & Ayoub 2011; Sobana & Panda 2011). In essence, the concentration at the membrane boundary layer is higher than the salt concentration in the feed. This is known as concentration polymerisation and will be discussed in more detail in Section 2.1.8.

2.1.6 Rejection

The rejection of a species is defined as the percentage of the influent concentration of a species that the membrane can retain. The rejection of a species can be calculated as follows (Kucera 2010):

$$\%\ Rejection = \frac{C_F - C_P}{C_F} \times 100 \quad \textbf{Equation 2-7}$$

where C_P is the concentration of a species in the permeate.

The average feed concentration is used in this calculation and not the concentration at a specific point on the membrane. The opposite of rejection is salt passage. Salt passage is defined as (Kucera 2010; El-Dessouky & Ettouney 2002):

$$\%\ Salt\ Passage = 100 - \%\ Rejection \quad \textbf{Equation 2-8}$$

$$\%\ Salt\ Passage = \frac{C_P}{C_F} \times 100 \quad \textbf{Equation 2-9}$$

Different factors influence the rejection of salt by a membrane. These factors include the ionic charge, the valency of the ion, molecular weight, polarity, molecular branching, hydration and degree of dissociation (Kucera 2010).

It should also be noted that a membrane does not have the ability to prevent salts completely from passing through the membrane. The rejection observed is from the difference in diffusion rates through the membrane. From Equation 2-5 and Equation 2-6 it is clear that only the water flux through the membrane is dependent on the operating pressure, while the salt passage is dependent on the concentration of salt and not the pressure (El-Dessouky & Ettouney 2002).

2.1.7 Data normalisation

Data normalisation is used to compare membrane performance at different times by eliminating the influence of temperature, pressure and concentration on the performance. This is done by normalising the data according to the design values. The performance of the membrane will then only be influenced by scaling, fouling or membrane degradation (Kucera 2010).

(a) Normalised flow

Normalised flow is the product flow without the influence of temperature, pressure and salt concentration. The normalised flow can be calculated as follows (Kucera 2010):

$$Normalized\ flow = \frac{[(AAP_s - \Delta\pi_s)]TCF_s}{[(AAP_a - \Delta\pi_a)]TCF_a} \cdot Actual\ flow \qquad \textbf{Equation 2-10}$$

where

AAP = Average trans-membrane pressure

$$= P_{Feed} - \frac{\Delta P}{2} - P_{Perm}$$

P_{Feed} = Inlet pressure

ΔP = Pressure drop over membrane surface

P_{Perm} = Permeate pressure

$\Delta\pi$ = Differential osmotic pressure across membrane

TCF = Temperature correlation factor

Subscript

"s" = Standard condition

"a" = Actual condition

The TCF is calculated as follows (Dow 2013):

$$TCF = \exp\left(2640 \times \left[\frac{1}{298} - \frac{1}{273+T}\right]\right); T \geq 25^oC$$

$$TCF = \exp\left(3020 \times \left[\frac{1}{298} - \frac{1}{273+T}\right]\right); T \leq 25^{o}C \quad \textbf{Equation 2-11}$$

where T is the temperature.

It is relatively easy to observe trends in the normalised flow once the data have been plotted (Kucera 2010).

(b) Normalised salt passage

Variations in inlet salt concentration and temperature will cause a variation in the salt passage through the membrane. It is therefore also necessary to normalise the salt passage to counter the influence of concentration and temperature (Kucera 2010). The normalised salt passage can be calculated as follows:

$$\%\ Normalized\ Salt\ Passage = \left[\frac{EPF_a}{EPF_s} \cdot \frac{CFC_s}{CFC_a} \cdot \frac{STCF_a}{STCF_s} \cdot \frac{C_{fa}}{C_{fs}}\right] \cdot \%SP_a \quad \textbf{Equation 2-12}$$

where

EPF = Average permeate flow divided by number of membrane modules

STCF = Salt transport temperature correction factor

C_f= Salt feed concentration

%SP = Salt Passage

CFC = Concentration of feed-concentrate

$$CFC = C_f \times \frac{ln\frac{1}{1-Y}}{Y}$$

$$Y = \frac{Product\ Flow}{Feed\ Flow}$$

Subscript

"s" = Standard condition

"a" = Actual condition

(c) Normalised pressure drop

The normalised pressure drop serves as indicator of when cleaning is necessary. This pressure drop is caused by fouling or scaling on the membrane. Severe fouling or scaling will cause irreversible damage to the membrane (Kucera 2010). The normalised pressure drop can be calculated as:

$$Normalized\ Differential\ Pressure = \frac{\Delta P_a \times (2 \times CF_{su} + PF_{su})^{1.5}}{(2 \times CF_a + PF_a)^{1.5}} \quad \textbf{Equation 2-13}$$

where

ΔP_a= Actual differential pressure

CF = Concentrate flow

PF = Permeate flow

Subscript

"su" = Start up

"a" = Actual condition

2.1.8 Concentration polarisation

A boundary layer is formed at the surface of the membrane when water flows over the membrane surface. The flow in the boundary layer is diffusion driven while the bulk flow is convection driven. The migration of water through the membrane causes convective flow to the membrane while the flow of salts to the bulk solution is diffusion-driven only. A higher salt concentration at the surface of the membrane than in the bulk flow is then observed. This is known as concentration polarisation (Kucera 2010). Concentration polarisation is illustrated in Figure 2-4.

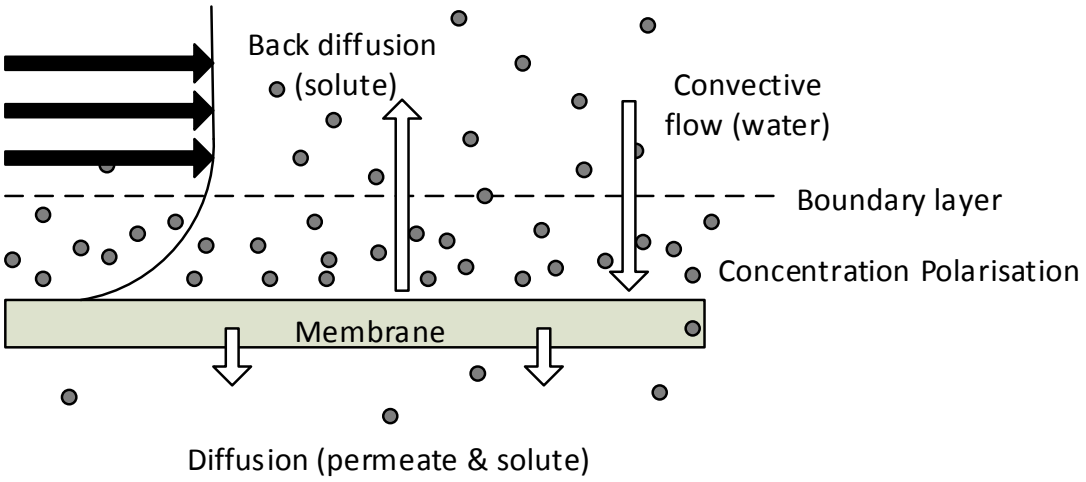


Figure 2-4: Concentration polarisation (redrawn from Goosen et al. 2004)

The effect of concentration polarisation can be decreased by e.g. increasing the cross-flow velocity over the membrane, therefore decreasing the boundary layer. Concentration polarisation is an unwanted phenomenon, responsible for a decrease in permeate flow and quality. The undesired impact of concentration polarisation is as follows (Kucera 2010; El-Dessouky & Ettouney 2002; Fritzmann et al. 2007):

- A higher osmotic pressure is required, since the concentration of salts is higher at the surface than in the bulk solution.
- Water flow through the membrane experiences hydraulic resistance, causing a decrease in permeate.

- A higher salt passage than expected since concentration of salts is higher at the membrane surface.
- Increased chance of precipitation of salts on membrane surface.
- Accumulation of particles on the membrane surface.

2.1.9 Fouling

Membrane fouling is an unwanted process in which materials accumulate on the surface of the membrane. The materials include colloidal particles, organic matter, inorganic matter and microbial organisms (Moonkhum et al. 2010; Kucera 2010). The deposition of these materials causes a decline in flux, an increase in required operating pressure and a decrease in product quality (Baker 2004).

(a) Colloidal/particulate fouling

Particulate matter has been classified by Rudolf and Balmat (1952) in four different categories, depending on the size of the particulates. The categories are as follows (Rudolfs & Balmat 1952):

Settleable solids	>100 µm
Supra-Colloidal solids	1 µm-100 µm
Colloidal solids	0.0001 µm (10$\dot{A}$) to 1 µm
Dissolved solids	<10$\dot{A}$

Colloidal fouling is the accumulation and sedimentation of particles and macromolecules on the surface and in the feed channel (Moonkhum et al. 2010). Colloidal fouling includes alumina-silicates, iron-silicates, clay and iron corrosion (Kucera 2010). Silica can settle out due to agglomeration in the presence of iron or aluminium even when the concentration of silica is below the saturation limit (Paul & Abanmy 1990; Potts et al. 1981).

(b) Organic fouling

Organic fouling is the adsorption of degraded organic materials such as plants that create a gel-like layer on the surface of the membrane. Humic and fulvic acids are among the macromolecules produced by plants (Fritzmann et al. 2007). This will then cause a flux decline and an increase in pressure drop over the membrane (Redondo & Lomax 1997).

(c) Inorganic fouling/scaling

Inorganic fouling is usually caused by Ca^{2+}, Mg^{2+}, CO_3^{-2}, SO_4^{-2}, silica and iron. The fouling is caused by the precipitation of the salts when the saturation limit is reached. $CaCO_3$, $CaSO_4$, $MgCO_3$ and silica depositions then occur. This is also known as scaling. Scaling/inorganic fouling is aggravated by an increase in flux, high recovery and low cross-flow velocity. Scaling normally occurs in the last stages of a RO system where the concentration of inorganic solutes is the greatest. Scale formation

also causes a higher operating pressure, higher than usual salt passage and higher pressure drop (Potts et al. 1981; Kucera 2010).

(d) Biological fouling

Biological fouling is the growth of microbes on the surface of the membrane, feed spacer or any other surface where conditions are favourable for microbial growth. A nutrient-rich environment is provided at the membrane surface by means of concentration polarisation. Microbes can also detach from the surface and migrate throughout the system to form secondary colonies elsewhere. Microbial growth is accompanied by the formation of a biofilm, which glues the cells together and to the surface, and protects the microbes against chemical treatment and shear forces (Kucera 2010; Potts et al. 1981 & Costerton et al. 1987).

2.1.10 Fouling potential of feed water

There are a number of different parameters used to measure the potential of the feed water to foul the membrane. Ensuring that the values of these parameters are below the recommended levels will not necessarily prevent fouling, although these values could provide an indication of the fouling potential of the feed stream.

(a) Silt density index

The silt density index (SDI) is an indication of the potential of the feed water to foul the membrane with colloids and suspended solids, especially colloids like alumina or iron silicates, clays, microbes, and iron corrosion products. The SDI is determined by the filtration time after sequentially passing two samples of influent through a 0.45-micron filter pad.

In brief, the time is taken to collect 500 ml filtrate through a 0.45-micron filter pad at a pressure of 2.07 bar (30psi). The influent is then allowed to run through the filter for 15 min at the same pressure. The time to collect another 500 ml sample is then recorded. The SDI is then determined as follows (Kucera 2010):

$$SDI = \frac{1-t_0/t_n}{n} \cdot 100 \qquad \textbf{Equation 2-14}$$

Where

t_o = Time to collect first 500 ml influent water

t_n = Time to collect 500 ml influent water after time n

n = Total run time (time between the two samples)

The colour on the filter pad is indicative of the type of potential foulant:

- Yellow: possibly organics or iron
- Red to reddish brown: Iron
- Black: Manganese (the colour dissolves when pad is treated with acid)

The SDI should usually not be more than a value of 5 not to void membrane warranty, but a value less than 3 is recommended (Kucera 2010).

(b) Modified fouling index

The modified fouling index (MFI) was developed by Schippers and Verdouw (1980) to overcome the deficiencies of the SDI (Schippers & Verdouw 1980).

The MFI gives a better linear correlation between the particle concentration and the observed fouling. The MFI is determined on the same equipment as the SDI and is based on cake formation, which occurs during filtration. It is determined by measuring the filtered volume every 30 seconds for a maximum time of 20 minutes. The MFI can be calculated as follows:

$$MFI = \frac{\mu_{20}}{\mu_T}\frac{\Delta P}{\Delta P_0} \cdot \tan(\alpha) \qquad \textbf{Equation 2-15}$$

where

μ_{20} = viscosity at 20 ^{o}C

μ_T = viscosity at water temperature

ΔP = transmembrane pressure at 20 ^{o}C

ΔP_0= Reference applied pressure (2.07 bar)

tan(α) = slope from the linear part of the plot [d(t/V)/dV]

The t/V relationship is plotted against the total filtered volume (V). The linear part of the curve represents the cake or gel formation and is used to determine the MFI. This is illustrated in Figure 2-5.

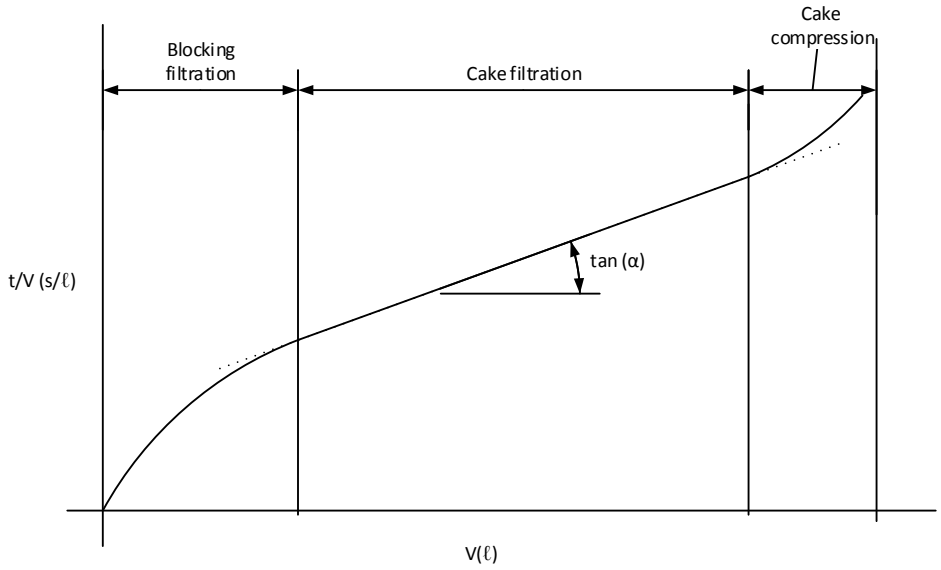


Figure 2-5: Ratio of filtration time and filtration volume (V) as a function of total filtration volume (redrawn from Schippers & Verdouw 1980)

A MFI from 0-2 s/litre^2 is recommended for RO and 0-10 s/litre^2 for nanofiltration (Fritzmann et al. 2007). The measurement of the MFI is a bit more complicated than the SDI. This makes the MDI less suitable to measure on a frequent basis (Alhadidi et al. 2011).

(c) Langelier saturation index

The Langelier Saturation Index is used to determine the potential of the influent water to scale or corrode. It takes pH, temperature, TDS, calcium hardness and alkalinity into account. The LSI can be calculated as follows:

$$LSI = pH - pH_a \qquad \textbf{Equation 2-16}$$

where

$$pH_a = (9.30 + A + B) - (C + D) \qquad \textbf{Equation 2-17}$$

where

A = (Log_{10}[TDS]-1)/10, where [TDS] is in ppm

B = -13.12 xLog_{10}(^oC + 273) + 34.55

C = Log_{10}[Ca^{2+}]-0.4, where [Ca^{2+}] is in ppm $CaCO_3$

D = Log_{10}[alkalinity], where alkalinity is in ppm $CaCO_3$

The water is prone to form calcium carbonate scale if the LSI is higher than 0. An LSI value of 0 is indicative of water that is in chemical balance, while a value below 0 will indicate that the water is

corrosive. The LSI can only be used for TDS values of up to 4 000 ppm. The Stiff-Davis Saturation Index (SDSI) is then used for higher TDS values:

$$SDSI = pH - pCa - p_{alk} - K \qquad \textbf{Equation 2-18}$$

where

pCa = ─Log10[Ca^{2+}], where [Ca^{2}] is in ppm

p_{alk} = ─Log10[total alkalinity], where alkalinity is in ppm

K = constant based on the total ionic strength and temperature

A LSI and SDSI value higher than 0 is an indication that the feed water has a tendency to form calcium carbonate scale. Softening is required as pretreatment or the use of acid and/or antiscalants are necessary (Kucera 2010).

(d) Calcium carbonate precipitation potential

The calcium carbonate precipitation potential (CCPP) provides quantitative measure of the degree of super-saturation of calcium carbonate. CCPP is proportional to the thermodynamics of precipitation, thereby estimating the amount of calcium carbonate that may dissolve or precipitate before reaching saturated condition. LSI, on the other hand, is only used to assess the saturation limit of calcium carbonate (Juby 2008; Rossum & Merrill 1983).

Table 2-1: State of CCPP corrosivity (adapted from Gebbie, 2000)

Corrosivity of the water	CCPP value (mg/L $CaCO_3$)
Scaling	> 0
Passive	0 to -5
Mildly Corrosive	-5 to -10
Corrosive (aggressive)	< -10

A drawback of CCPP is, however, the tedious and time-consuming calculations necessary for the determination of the value when a computer-assisted program is not used.

(e) Turbidity

Turbidity is used as a measure of the amount of suspended solids. It measures the light scattering ability of the particles in the water. Turbidity is measured in Nephelometric Turbidity Units (NTU). Influent water with turbidity less than 0.5 NTU is recommended. It should also be noted that there is no direct correlation between turbidity and SDI (Kucera 2010).

(f) Colour

The true colour or apparent colour of the influent stream can also be measured. The apparent colour is the colour measured when there are still dissolved and suspended particles in the solution, whereas the true colour is the colour when all the suspended solids are filtered out. The APHA (American Public Health Association) dimensionless units are used to measure colour. A value of less than 3 is recommended (Kucera 2010).

(g) Organics

Organic substances can be measured as total organic carbon (TOC). The recommended TOC should be less than 3 ppm and the concentration of oils (both silicone and hydrocarbon based) should be less than 0.1 ppm. This is only an indication and does not guarantee that no organic fouling will take place (Kucera 2010).

(h) Microbes in feed water

Assimilable organic carbon (AOC) in the influent water can be used as an indication of the biological fouling potential of the feed water. The number of colony forming units (CFU) in the feed stream can also be measured, although only 1-10% of bacteria are culturable on laboratory culture media (Kucera 2010). However, this remains an inexpensive technique for tracing microbial fouling. Less than 100 CFU/ml is recommended. The total bacterial count can also be used (TBC). A water sample is filtered and the microbes on the filter viewed using epifluorescence staining techniques. This is a quick, but not always a practical technique, since the microscope itself and the staining kits are very expensive. Microbes in the feed water are discussed in more detail in Section 2.2.6.

2.1.11 System designs

As discussed in Section 2.1.3, RO membranes are packed in spiral-wound modules. These modules cannot withstand the operating pressures required on their own. These modules are then placed inside pressure vessels to handle the operating pressures. Interconnectors are used to connect the spiral-wound elements with each other inside the pressure vessel. The pressure vessels are then arranged in different configurations to make up multiple stages as shown in Figure 2-6. The design used will depend on the requirement of the plant (Schwinge et al. 2004).

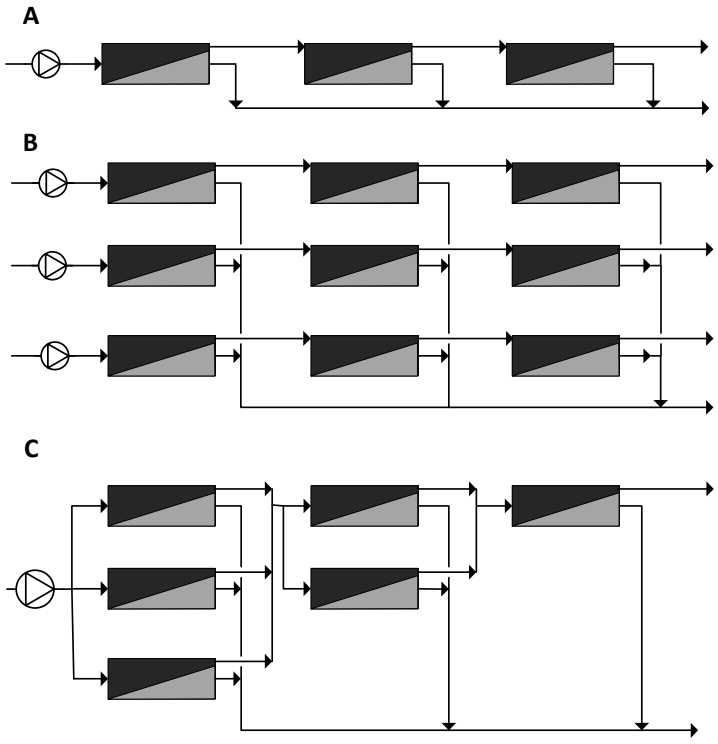


Figure 2-6: Plant configurations used in reverse-osmosis plants. (A)-Series array. (B)-Parallel array. (C)-Tampered array (redrawn from Fritzmann et al. 2007)

The simplest system design is the series array configuration (Figure 2-6 (A)). A number of elements are connected in series. The maximum housing length is limited by the pressure drop along the feed channel and the fouling potential of the water. A plant with higher throughputs will make use of multiple stages in parallel (Figure 2-6 (B)). When fouling and concentration polarisation becomes significant, a tapered design can be used as shown in Figure 2-6 (C). The decrease in number of modules in each stage will boost the velocity of the feed water. A high recovery can still then be achieved by avoiding the worst effects of fouling and concentration polarisation (Greenlee et al. 2009; Fritzmann et al. 2007; Kucera 2010).

The fouling types encountered throughout the system are also not constant. Biological fouling will usually be more dominant during the first stage while scaling occurs at later stages (Kucera 2010). It is therefore not always easy to simulate fouling on a single flat-sheet membrane.

2.2 Biofilm formation on membranes

2.2.1 Characteristics of a biofilm

Adequate pretreatment of the feed water to reverse-osmosis membranes can reduce the colloidal/particular, inorganic and organic fouling significantly. This is, however, not the case with

biological fouling; even if only a few bacteria cells survive the pretreatment (0.01% of total cells), they are still able to adhere to the surface, reproduce and then relocate to different parts in the membrane module (Matin et al. 2011; Flemming et al. 1997).

Biofouling not only affects RO membranes, but also nanofiltration (NF), ultrafiltration (UF) and microfiltration membranes (MF). Undesired effects from biofouling include (Nguyen et al. 2012):

- Increased energy consumption for the higher than usual feed pressure required.
- Increased degree of concentration polarisation due to the accumulation of salts in the biofilm. This will increase salt passage and reduce the permeate quality.
- Membranes (especially cellulose acetate membranes) are vulnerable to acidic by-products at the membrane surface, formed by the biofilm, which causes membrane degradation.
- A lower flux due to blockage from biofilm.
- Shortened membrane life.

2.2.2 Transport and attachment of microorganisms

Microorganisms that were not removed in the pretreatment process are transported to the vicinity of the membrane surface by standard fluid dynamic forces. The feed spacer that provides turbulence in the feed channel also creates stagnant zones with low flow velocities (Radu et al. 2010). Only a change in flow pattern will remove matter caught up in the stagnant zone. Other forces (for example, Brownian motion) operate in these stagnant zones, which helps with the transportation of non-motile cells to the membrane surface. Motile cells exhibiting chemotaxis will migrate to the surface of the membrane in response to the higher concentration of nutrients (Wilbert 1997).

The microorganisms can attach to a surface, called the substrate, which is either clean or covered with a conditioning film (Al-Juboori & Yusaf 2012). Such a conditioning film is usually formed when a surface is covered in water. Figure 2-7 is a representation of a gram-negative cell approaching the substrate covered with a conditioning film. The conditioning film consists of biopolymers and proteinaceous molecules deposited onto the membrane during filtration and it provides a large number of binding sites for cells through hydroxyl groups, polarised bonds and charged groups. Original surface properties are also to some extent masked by the conditioning film (Al-Juboori & Yusaf 2012; Flemming et al. 2011) where on pristine surfaces, the adhesion is mainly covered by the macroscopic properties of the surface (Busscher et al. 2010).

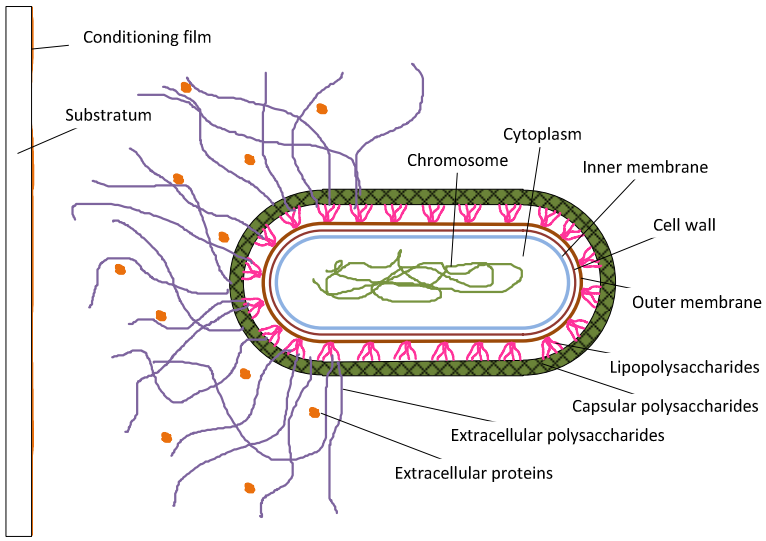


Figure 2-7: Gram-negative bacterium approaching a submerged surface (redrawn from Flemming 2011)

A number of different forces are responsible for the adhesion of the microorganisms to the surface. The forces responsible are shown in Figure 2-8. These forces are (Al-Juboori & Yusaf 2012):

Hydrodynamic forces: Hydrodynamic forces are responsible for bringing microorganisms within close range with the surface. These forces include the diffusive and convective forces resulting from water passing over the RO membrane. Adhesion will be facilitated by the following three interactions once the microorganisms are close to the surface.

Physio-chemical interaction*:* These are the interactions between the microorganisms and the membrane surface dominated by Lewis acid-base, Liftshitz-van der Waals interactions and the electrostatic double layer of the cell and surface. Initial cell adhesion (facilitated by physio-chemical interactions) to the surface is followed by secondary adhesion, where the new cells attach via cohesion to the already attached cells (Brant & Childress 2002).

Ligand-receptor interactions*:* These are the interactions between the receptors of the microorganisms and binding site created by the conditioning film, which include polarised bonds, hydroxyl groups or charges groups (Flemming et al. 2011).

Adhesive interactions*:* Interactions exist between the appendages of some microorganisms in the bulk liquid and the sticky nature of the Extracellular Polymeric Substances (EPS). These interactions can help cells anchor itself into the membrane surface. The water film between the membrane surface and the cell can also be removed by the appendages, allowing the cells to bind directly to the surface.

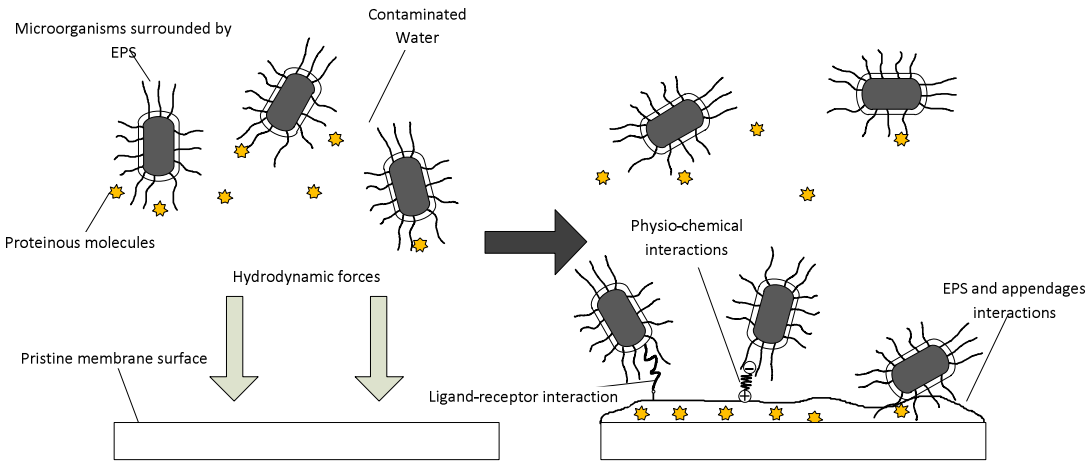


Figure 2-8: The driving forces responsible for microbial adhesion (redrawn from Al-Juboori & Yusaf 2012)

2.2.3 Factors influencing microbial adhesion

There are different factors that have an influence on the adhesion of microorganisms on the surface of the membrane. Table 2-2 summarises the factors that influence microbial adhesion and are discussed afterwards.

Table 2-2: Factors affecting microbial adhesion (Al-Juboori & Yusaf 2012; Flemming & Schaule 1988)

Factors influencing microbial adhesion	Composition of factors
Mass transport conditions of liquid	Shear forces (flow)
	Boundary layer
	Flux (vertical forces)
	Surface tension
Solution ionic strength	Dissolved inorganic substances
	Suspended matter
pH of solution	Electrostatic double layer
Surface characteristics	Chemical composition
	Surface charge
	Surface tension
	Hydrophobicity
	Conditioning film
	Roughness
	Porosity
Nutrient concentration	Nutrient status
Microorganisms	Species
	Composition of mixed population
	Growth Phase
	Population density

Mass transport conditions of liquid: The flow of liquid affects the accumulation rate of microorganisms and nutrients at the membrane surface. The turbulence in the flowing liquid can also affect the shear force on the membrane surface, influencing microbial adhesion to the membrane and the thickness of the biofilm formed (Ying et al. 2013). A high shear rate can decrease the rate of biofilm formation, but may also lead to biofilms that are mechanically more stable.

Solution ionic strength: The ionic strength of the solution can affect the electrostatic double layer interaction between the microorganisms and the membrane surface. A solution with a lower ionic strength will cause the energy layer to increase, thereby increasing the repulsive energy of the electrostatic double layers' interaction between the microorganisms and the membrane surface. The cells then find it difficult to penetrate the energy layer using EPS and nanofibers, thus decreasing the amount of biofouling (Hori & Matsumoto 2010).

pH of solution: The acidity of the solution affects the surface charge of the membrane and the microorganisms, which in turn can affect the electrostatic double-layer interactions between the microorganism and the membrane. Brant and Childress (2002) have found that the pH affected the surface charge of the colloids much more that of the membrane (Brant & Childress 2002).

Surface characteristics: Microbial adhesion is greatly influenced by the surface characteristics of the membrane. Surface characteristics include surface roughness, hydrophilicity and hydrophobicity. The tendency of the membrane to foul is characteristics of the hydrophobicity and hydrophilicity of the membrane. Membrane roughness was also found to promote microbial adhesion to the surface since sites that are more suitable are provided for adhesion (Vrijenhoek et al. 2001).

Nutrient concentration: Bulk solutions rich in nutrients stimulate microbial growth and metabolic activity, leading to an increase in biofoulant mass. The nutrients in the water can come from existing contaminants in the feed water or leach from the substratum (Kucera 2010).

Microorganisms: A higher concentration of microorganisms in the feed water will promote the adhesion rate of microbial organisms to the membrane surface. However, Vrouwenvelder et al. (2008) found no relationship between the concentration of microorganisms, measured with total direct count (TDC), in the feed and the amount of biofouling on the membrane (Vrouwenvelder et al. 2008). The concentration of microorganisms will therefore only influence the adhesion rate; it cannot be used to quantify the amount of biofouling on the membrane. The type of microorganism will also influence the adhesion rate. Some types of microorganisms take several days to reach a specific surface concentration where other strains only take a few hours (Flemming & Schaule 1988).

2.2.4 Membrane colonisation

The biological fouling of a membrane occurs in a sequence of events. The events include (1) the transportation of microorganisms to the membrane, (2) the adhesion and attachment of the microorganisms to the membrane surface, (3) the growth of microorganisms on the membrane, which includes the secretion of EPS followed by the dispersion to other locations on the membrane (Flemming & Schaule 1988; Matin et al. 2011). This sequence of events is shown in Figure 2-9.

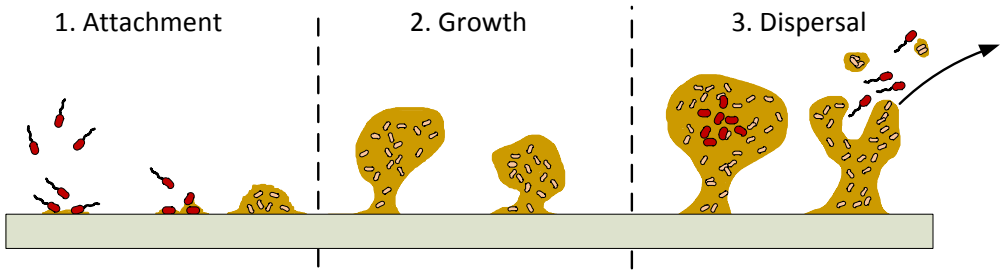


Figure 2-9: Biofilm formation sequence (redrawn from Matin et al. 2011)

The primary adhesion of the microorganism to the surface is shown in Figure 2-10 (step 2 of the colonisation process). The flux and flow are some of the hydrodynamic forces that influence the adhesion of the microorganism. The adhesion is also assisted by the fimbriae. The flux and flow assist the cells with transportation to the vicinity of the membrane surface before adhesion can occur.

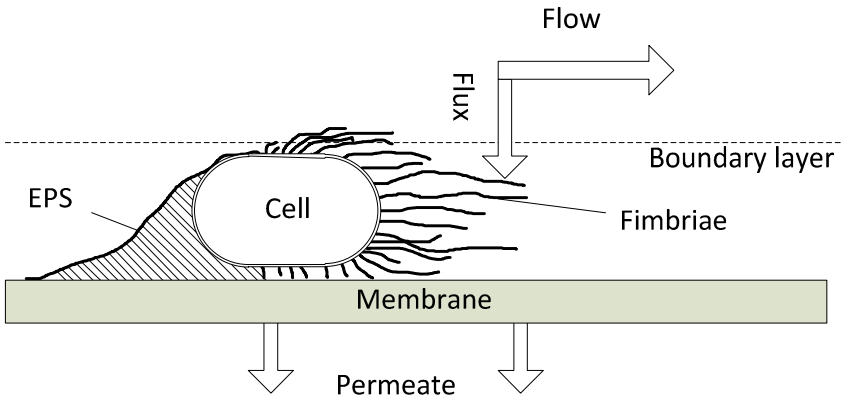


Figure 2-10: Primary adhesion of microorganism (redrawn from Flemming & Schaule 1988)

The transport and attachment of microorganisms to the surface are influenced by many factors that have already been discussed. Micro-colonies are then formed by the aggregation of microorganisms on the membrane surface (Ramsey & Whiteley 2004). The formation of micro-colonies is followed by the production of extracellular polymeric substances (EPS). EPS acts as a protective jell around the cells. During the disposal phase, sub-populations detach from the mature biofilm to colonise different parts of the membrane. The process then starts from the beginning (Al-Juboori & Yusaf 2012).

2.2.5 Extracellular polymeric substances

Extracellular polymeric substances (EPS), also known as Transparent Exopolymer Particulate (TEP), are secreted during the growth and reproduction of microorganisms (Komlenic 2010). EPS is a complex, high-molecular weight mixture of polymers that consists of proteins, polysaccharides, nucleic acids and lipids. The EPS influences the primary adhesion of microorganisms to a solid surface by altering the physiochemical characteristics of the microorganisms such as the hydrophobicity and surface charge. The EPS also provide a structure to the microorganisms, allowing cells to bind together in an EPS matrix. A relationship then forms between the EPS and microorganisms, which influences several properties of the microbial aggregates. The properties are listed below.

These properties include (Sheng et al. 2010):

- Mass transfer
- Surface charge
- Flocculation ability
- Ability to settle
- Dewatering ability
- Stability
- Adhesion ability
- Formation of aggregates

Once the EPS matrix is bound to the membrane surface, the adhesive properties trap bacteria, viruses and inorganic particles that are present in the feed water. The EPS can also provide a source of food for the metabolic activities of the cells when there are insufficient nutrients in the feed water (Komlenic 2010). The ability of EPS to absorb water, provide stability and influence the mass transfer to the cells also protects the cells from toxic substances. These toxic substances can include biocides, which are used to control biofouling, making it difficult to remove the fouling layer easily.

EPS can be divided into two groups. These two groups are bound EPS, including capsular polymers, loosely bound polymers and condensed gels and soluble EPS, which include slimes and colloids (Laspidou & Rittmann 2002; Jiao et al. 2010). A strong bond exists between the cells and the bound EPS, while only a weak bond exist between the soluble EPS and the cells, since the soluble EPS can also be dissolved (Sheng et al. 2010). Soluble EPS have the ability to accumulate on the surface of the membrane and penetrate into the pores, making it one of the most stubborn naturally occurring organic foulants of UF, NF and RO membranes (Meng et al. 2009).

The EPS (both bound and soluble) are primarily responsible for the decline in performance of the membrane, while the cells are responsible to a lesser extent. Accumulation of the EPS on the membrane causes a gel-like layer to form on the membrane by cross-linking with the membrane. This gel-like layer is rich in nutrients and promotes additional microbial adhesion to the membrane surface. The formation of the gel-like layer is influenced by different factors, which are listed below:

- Cohesion characteristics of EPS
- Adhesion characteristics
- Flexibility and rearrangement characteristics
- Morphology of membrane surface
- Diffusion of EPS into pores
- Flow pattern near surface

Some of these factors are also influenced by each other. Ying et al. (2013) have investigated the effect of shear rate, influenced by the flow patterns, on biofouling on reverse-osmosis membranes. It was found that shear rate influences the formation of the EPS chemical composition, which in turn corresponds to the change elasticity and cohesion of the EPS. These physical changes then also have different impacts on the flux decline (Ying et al. 2013). This means that the morphology of the EPS is influenced by the shear rate.

It has already been mentioned that the biofouling layer (consisting mostly of EPS) has an unwanted effect on membrane performance. This is caused by several factors related to EPS. Concentration polarisation is increased by reduced turbulent flow near the surface, which obstructs back-diffusion of solutes to the bulk fluid. The increase in solutes near the surface increases the localised osmotic pressure of the fluid (concentration polarisation) and decreases the rejection of the membrane. The void fraction between the cells is also reduced, decreasing the water permeation through the membrane. EPS further restricts the transportation of anti-microbial agents to the microorganisms in the biofilm (Flemming 1997).

Although some of the properties of the EPS are known, the exact function of the EPS is still unclear, given the heterogeneous composition of the material. In order to clarify the function of the EPS, the amount of the different components in the EPS and their influence on cell surface properties must be determined (Tsuneda et al. 2003). However, some of the factors (e.g. flow pattern and membrane surface), known to influence the formation of the biofilm, can possibly be manipulated to control microbial fouling better.

2.2.6 Microbial diversity on membrane surfaces

Different methods exist that can be used to determine the organisms in a microbial community. These methods can be divided into culture-based and culture-independent methods. Culture-based methods rely on the cultivation of the microorganisms on media in a laboratory before identification. During culture-independent methods, DNA is extracted directly from the microorganisms.

The true composition is not always reflected by culture-based methods, because only a small fraction of the biological community found on membrane surfaces and in a membrane spacer, can be cultivated in a laboratory (Schut 1993). It is therefore necessary to use a combination of culture based and culture independent methods to determine the microbial species that are living in an RO system.

The variations in the bacteria community are influenced by a number of factors. The factors are listed in Table 2-3 and discussed below.

Table 2-3: Factors influencing the microbial community on a membrane surface

Factors	Description
Feed water	The source of feed water will influence the types of microorganisms encountered, e.g. brackish, seawater or potable water, as well as the pretreatment process and concentration of contaminants in the water, e.g. chemicals in the feed and nutrients.
Location in plant	Different microorganisms are encountered in different sections of the plant e.g. feed water, cartridge filters, UF and RO membranes. The different RO systems are discussed in Section 2.1.11.
Hydrodynamic forces	The shear rate on the membrane influences the microbial community.

There is a large difference between the dominant microorganisms found on the membrane surface during seawater desalination compared to brackish water desalination (Zhang et al. 2011). Bereschenko et al. (2008) constructed a clone library of microorganisms found on a membrane surface with fresh water as feed. In brief, genes were extracted from the microorganisms and cloned into host organisms. The clones were grouped together in clone families. From these families, a clone with a unique sequence was selected of which the full sequence of the 16S rRNS was determined to classify the microorganisms. In this study they found that 59% of the clones were related to the *Proteobacteria* class, which was dominated (27% of all clone) by *Sphingomonas spp.* (Bereschenko et al. 2008). Khambhaty and Plumb (2011) also found similar results for the bacterial community on RO membranes used for brackish water desalination. They identified species affiliated with γ - *Proteobacteria*, which are mainly comprised of *Pseudomonas* and *Xanthomonas* followed by *Sphingomonas* (Khambhaty & Plumb 2011). The microorganism community on seawater RO membranes was found to be dominated by *Leucothrix mucor*, which accounts for 30% of the clone library used. The rest of the microorganisms (61.25% of total clones) belonged to the

Alphaproteobacteria family (Zhang et al. 2011). It is clear that variations exist between the micro-organism communities found on RO membranes with different feed-water sources. Using a single microbial strain might therefore not provide a sufficient representation of the behaviour of the organisms on the membrane.

A variation in microorganisms at the different stages of the purification process also exists with the different feedwater sources (seawater or brackish water). This means that the composition of the microorganism communities found in the feed, ultra-filtration, cartridge filter, RO membrane and product stream are all different (Bereschenko et al. 2008). Zhang et al. (2011) found variations in the microbial community found in the cartridge filter compared to the RO membrane during seawater desalination. Bereschenko et al. (2008) also found in their study that the relative abundance of different species in the biofilm was different from those in the feed. This indicates that the biofilm on the membrane were actively formed and not a result of the bacteria concentration in the feed. This active formation of the biofilm can explain the variations observed in the different parts of the system.

Al Ashhab et al. (2014) found that the shear rate (corresponding to the cross-flow velocity) had an influence on the microbial community composition during tertiary wastewater desalination. The microbial diversity was the highest when a medium shear rate was applied, compared to high and low shear rates. *Betaproteobacteria* dominated medium shear rates while *Alpha-* and *Gamma-Proteobacteria* dominated the low and higher shear rates. A higher number of bacteria are also observed with the low and medium shear rates compared with the higher shear rates. Bacteria known to secrete a high amount of EPS were found to be more abundant during the higher shear rates (Al Ashhab et al. 2014).

It should be noted that biofouling is not limited only to bacteria, but can also consist of algae, fungi and protozoa (Characklis 1991). The physiological differences between these different species are shown in Table 2-4. Larger organisms (e.g. protozoa, larger algae, and fungus cells) can be removed relatively easily from the feed water with filtration (Wilbert 1997).

Table 2-4: Physiological differences between microorganisms (adapted from Wilbert 1997)

	Microalgae	**Fungi**	**Protozoa**	**Bacteria**
Size	Multi-or unicellular	2-5 µm-diameter cells forming mycelium	5 µm-1 mm	0.5-3 µm 0.2-0.5 µm starved
Life Cycle	Free-living/plant-like	Spore/mycelium or motile cells/ mycelium	Free-living or parasitic, with encasement	Sporulation after active reproductive cycle
Metabolism	Photosynthetic	Simple carbohydrates, some produce enzymes to utilise complex carbon	Omnivorous	Photosynthesis, oxidation of inorganic, or carbon with/without oxygen

Reproductive Rate	Asexual fission or spores, depending on conditions	Vegetative from hyphal fragments, sexual/asexual spores	Nutrient level dependent	0.3-15 hour mean generation time, dependent on nutrient level
Attachment	With acidic polysaccharide mucilaginous materials	Rhizoid holdfasts	With adhesive holdfast appendages, suction mechanisms	Hydrophobic interactions, surface appendages
Motility	With flagella or free drifting	Reproductive cells	Flagella, pseudopodia, cilia, or non-motile with spores	Some do
EPS	No	Yes	No	Some do
Cell Composition	Cellulose, diatoms with silica	Chitin-cellulose	Some have calcareous or silica shells	Phospholipids, protein peptidoglycan

The microorganisms found on membrane surfaces are not limited to the bacteria mentioned above. Some of the more abundant microorganisms found (but not limited to) on membrane surfaces together with the characteristics of some of the microorganisms are listed in Table 2-5.

Table 2-5: Microorganisms found on membrane surfaces with different feed-water sources with some prominent microorganism characteristics

Water Source	Microorganisms	Characteristics of some of the bacteria	Reference
Fresh Water	*Sphingomonas spp., Microbacterium spp., Rhodopsedumonas sp.,Lysobacter spp., Nitrosomonas spp.*	Biofilm formation is initiated and dominated by *Sphingomonas spp.*	(Bereschenko et al. 2008; Bereschenko et al. 2010; Pang et al. 2005)
Brackish water	*Pseudomonas, Xanthomonas, Sphingomonas spp.,*	*Pseudomonas spp.* has biofilm forming ability, therefore used as model organism.	(Khambhaty & Plumb 2011; Khambhaty & Plumb 2011; Barnes et al. 2014)
Sea water	*Leucothrix mucor,Ruegeria, Rhodobacteraceae*	*Leucothrix mucor* is filamentous	(Zhang et al. 2011; Lee & Kim 2011)

2.3 Monitoring and characterisation of biofouling

A biofilm will develop on any surface submerged in nonsterile water, but this does not mean the system suffers from biofouling. Biofouling only occurs when a certain threshold is reached, which is dependent on the specific system and will be determined by the economic impact, the product quality, or the overall system performance. The threshold is illustrated in Figure 2-11 (Flemming et al. 2011).

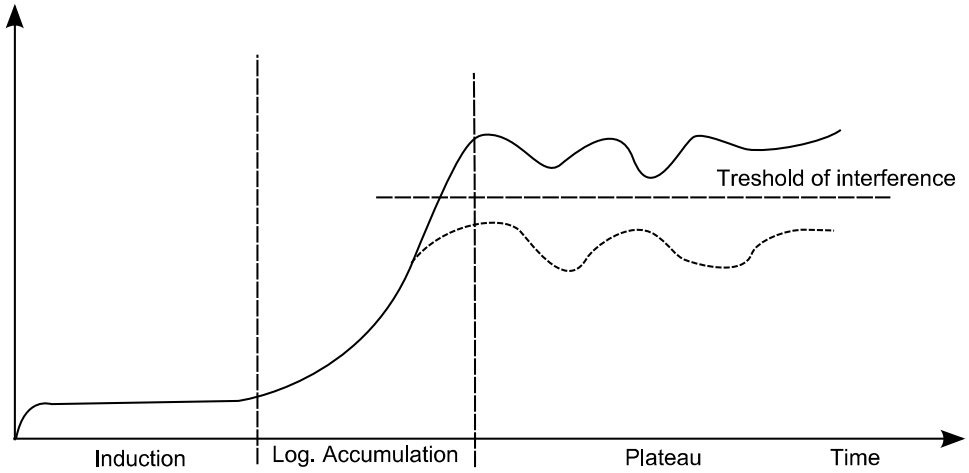


Figure 2-11: Biofilm development below and above threshold level (Flemming 1997)

The threshold is industry dependant, but is usually reached when product quality, efficiency or production is between 10% and 20% less than usual. Industry-based methods will then be used to deal with the problem, which is not always effective. The problem can be avoided when early warning systems are in place (Flemming et al. 2011).

2.3.1 Monitoring biofouling

There are many different techniques available to monitor the biological growth on membranes and fouling potential of the feed water. Monitoring focuses on feed water characteristics, biofilm growth and system performance (Flemming et al. 2011). A monitoring system must be able to indicate the extent, location, composition and kinetics of the deposition required to be effective. Measurements required for the system must be performed automatically, in-line, continuously, accurately, fast, reproducibly and in a non-destructive way (Klahre & Flemming 2000). Different methods, but not limited to that, are used to monitor the biofouling on the membranes are listed and discussed in Table 2-6.

Table 2-6: Methods to monitor biofouling

Detection Method	Description	References
Feed Water Biological Parameters	The biological fouling potential of feed water can be assessed by measuring the adenosine triphosphate (ATP) content in the water, the number of cells, assailable organic carbon (AOC) concentration and the biofilm formation rate (BFR). Sampling and analysis are required to determine these parameters. It is therefore not a suitable online method for biofouling detection, but can rather be used as an off-line early warning system.	(Van der Kooij 1992; Arnal et al. 2011; Nguyen et al. 2012)
System Performance	The pressure drop, permeate flux, oxygen uptake and salt passage can be used to characterise the biofilm on the membrane surface. Microorganisms utilise dissolved oxygen in the water for growth and metabolic activities. Online measuring of the oxygen uptake is not practical, since the microorganisms use a very small amount of oxygen for metabolic activities. Feed water can be circulated (e.g. for 2 h) to increase the oxygen consumption to a measurable value. This method is only able to determine the amount of active biomass on the surface.	(Vrouwenvelder et al. 2011; Brouwer et al. 2006; Vrouwenvelder et al. 2003)
Fluorometry	A fluorogenic agent (e.g. for pyranine phosphate or fluorescein diphosphate) is added to the feed water. The fluorogenic agent undergoes a fluorescent signal change when it makes contact with a wide range of microorganisms. An on-line fluorometer can then be used to monitor the change in fluorescent signal. Care should be taken when a fluorogenic agent is used, since pH and temperature may have an effect on fluorescent signal. The choice of fluorogenic agent must also not have a negative impact on the membrane materials, must be environmentally friendly and must not decrease the efficiency of pretreatment chemicals.	(Ho et al., 2004)
Silent Alarm™ system	The Silent Alarm™ is an innovative monitoring technique developed by MASAR Technologies, Inc. to detect and measure fouling in a RO and NF system. It can detect the fouling or scaling early by using a parameter know as Fouling Monitor (FM). The FM parameter is the percentage difference between the normalised data based on the ASTM D-4516 standard and the corrected normalised flow for each data point. A drawback of the Silent Alarm™ is that it cannot distinguish between biofouling and organic/scaling.	(Saad 2004)
Membrane Fouling Simulator	The membrane-fouling simulator (MFS) is also an early warning system for biofouling. For the MFS to operate it requires feed water also supplied to the RO/NF system, a sensitive differential pressure drop transmitter and a high cross-flow velocity to increase pressure drop and decrease time to detection. The differential pressure drop is only measured over the feed channel under no filtration conditions. A decline in flux caused by biofouling is therefore not measured.	(Vrouwenvelder et al. 2011)
Ultrasonic Time-Domain Reflectometry (UTDR)	UTDR are used for early-stage biofilm detection on membrane surfaces. Soundwaves are used to locate and determine biofilm-formation physical characteristics. Studies using this method have been limited to laboratory scale.	(Kujundzic et al. 2007; Mairal et al. 2000)
Electric Potential Measurements	The electric potential varies during cake layer formation. This variation can be used as biofilm indicator. The electrical potential is based on the properties of the cake layer, which can be influenced by the pressure. Electrical potential must therefore be measured at constant pressure to minimise the errors.	(Sung et al. 2003; Teychene et al. 2011)

Rotoscope	A disk is allowed to rotate through water. The light reflected from the surface changes as the biofilm develops. The amount of reflected light is then measured.	(Cloete & Maluleke 2005)
Fiberoptical Device	The light backscatter caused by deposits on top of light fibre is measured.	(Tamachkiarow & Flemming 2003)

It is clear from Table 2-6 that a wide variety of different methods exists that can be used to monitor the formation of biofouling on the membrane surfaces. A single method alone will not be enough to monitor and control biofouling completely. It is therefore recommended that a combination of the different tools available be used to monitor the growth of a biofilm better. This, however, will increase costs.

2.3.2 Biofilm characterisation

Equally, there are many different techniques to analyse the biofouling on the membrane surface. These techniques are normally destructive and are performed offline in a laboratory. There are mainly two branches of relevant analytical methods: microscopic techniques and spectroscopic techniques (Al-Juboori & Yusaf 2012).

(a) Microscopic techniques

Microscopic techniques can be used in a destructive or non-destructive way to analyse biofouling. The different microscopic techniques used to study biofilm formation are listed and discussed in Table 2-7.

Table 2-7: Different microscopic techniques available

Microscopic Techniques	Description	References
Light Microscopy	Relatively simple to use with little sample preparation required. Light microscopy provides basic information of the biofilm present on the surface. Light microscopy only provides preliminary analysis of the biofilm since it cannot provide information on the spatial distribution and depth of the biofilm.	(Wolf et al. 2002; Ridgway et al. 1984; Lazarova & Manem 1995)
Epifluorescence Microscopy	A sample is stained with fluorochrome prior to epifluorescence microscopy. The sample is then subjected to light with different wavelengths (visible/ UV light). Excited molecules in the biofilm emit light with a longer wavelength than the light supplied. Epifluorescence microscopy is also easy to use and provides useful information on the structure-function relationships of the biofilm.	(Wolf et al. 2002; Khan et al. 2010; Al-Juboori et al. 2012; Ridgway et al. 1984;Lemarchand et al. 2001)
Electron Microscopy	Electron microscopy consists of scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Electron microscopy can provide high-resolution images and the spatial distribution of the biofilm. However, sample preparation is difficult and it is not possible to do *in situ* and *in vivo* analyses.	(Wolf et al. 2002; Surman et al. 1996)

Confocal Laser Scanning Microscopy (CLSM)	CLSM is able to produce three-dimensional images of a biofilm by using a laser light that can penetrate deeply into the biofilm and excite certain components. These components can include fluorophore molecules added to the biofilm or naturally occurring molecules inside the biofilm. An advantage of the CLSM is that is can provide information on the metabolic activity and biofilm microenvironments. A drawback is the slow scanning speed of the CLSM and overlapping of signals.	(Herzberg & Elimelech 2007; Wolf et al. 2002)
Other microscopic techniques	There are still many different microscopic techniques that can be used to quantify the biological growth on the surface. Some of these microscopic techniques include atomic force microscopy, X-ray microscopy, Raman microscopy, Hoffman modulation contrast microscopy (HMCM), differential interference contrast microscopy (DICM), environmental scanning electron microscopy (ESEM), digital time-lapse microscopy and image analysis.	(Al-Juboori & Yusaf 2012)

Light microscopy is not always only used to characterise the biofilm, but also to count the number of cells in the feed stream and on the membrane. They can only count the number of cells and not distinguish between live and dead cells, except if they are motile. It is, however, still a practical and easy method, which has been used in a number of studies (Ridgway et al. 1984; Dreszer et al. 2014). The commercially available staining kits (e.g. LIVE/DEAD BacLight) that are used for epifluorescence microscopy and CLSM are very expensive. Nevertheless, they are still widely used to examine biofilm growth in laboratories (Yu et al. 2013; Suwarno et al. 2012; Dreszer et al. 2013)

(b) Spectroscopic techniques

Spectroscopic techniques have the potential to give qualitative and quantitative information about biofilm growth and accumulation. The different spectroscopic techniques that are commonly used are listed and discussed in Table 2-8.

Table 2-8: Spectroscopic techniques available

Spectroscopic Techniques	Description	References
Infrared Spectroscopy	A sample is exposed to infrared (IR) radiation, which causes the molecules to absorb the radiation. The absorption of radiation will cause the molecules to vibrate. The amount of radiation absorption is proportional to the amount of molecules present in the biofilm. IR is quick, accurate and can analyse samples in different phases. It is, however, limited to a thin biofilm and the effect of the refractive index of the surroundings must be known.	(Davis & Mauer 2010; Wolf et al. 2002)
Bioluminescence	Some organisms emit light when certain biochemical reactions occur. This phenomenon can be used to characterise the biofilm by determining bacteria biomass and cellular activity. However, the use of bioluminescence is limited to microorganisms that can emit light under biochemical reactions.	(Wolf et al. 2002; Stewart et al. 1992)

Photo-acoustic Spectroscopy (PAS):	PAS makes use of light and ultrasound to analyse attached molecules on solid surfaces. PAS is not suitable for RO membranes, but can be used in the piping prior to RO to determine the fouling. PAS can be used to determine the thickness of the biofilm and growth and detachment of the microorganisms from the surface. PAS cannot be used to determine the structure of the biofilm and provides low-resolution images.	(Bageshwar et al. 2010; Schmid et al. 2001; Schmid et al. 2002)
Fluorometry	Fluorometry can be used to monitor bacterial activity in a biofilm and characterise the structure. The biomolecules present in a sample is stimulated when subjected to UV or visible light. Different biomolecules will emit different fluorescence signals, which can then be used to characterise the biofilm. Fluorometry is a good tool to use for biofilm development. It can however be influenced by environmental factors such as pH, temperature and agitation.	(Janknecht & Melo 2003)
Nuclear magnetic resonance (NMR) spectroscopy	NMR is a non-destructive and non-invasive technique that can be used to obtain information about the metabolic pathways and the mass transfer phenomena in the biofilm. During NMR, radio frequencies are used to excite atoms in the biofilm, which in turn release radio frequencies. A disadvantage of NMR is the low signal/noise ration and the low quality of the images generated.	(Janknecht & Melo 2003; Serafim et al. 2002)

Excessive biofilm growth can make it difficult to quantify if with spectroscopic techniques (infrared spectroscopy) since these techniques are limited to thin biofilms. The wide variety of microorganisms also makes it difficult to use bioluminescence, since it is organism dependant. However, the range of different spectroscopic techniques available makes is possible that at least one of the techniques will likely be suitable for a required application, providing valuable biofilm information.

2.3.3 Lab-scale monitoring

Carrying out experiments on industrial scale can be very expensive and difficult due to the variability in feed conditions. Therefore, most of the setups used for biofouling testing are bench scale. A variety of feed organisms is fed to the cross-flow units with the feed water. The microorganisms in the feed range from a single microbial strain to artificially mixed cultures containing different known species and naturally occurring microorganisms with unknown composition, usually found in tap water (Suwarno et al. 2012; Dreszer et al. 2013; Goldman et al. 2009).

The normalised trans-membrane pressure, feed-channel pressure drop, normalised permeates flux and normalised salt passage will typically be monitored. Membrane autopsy is also relatively easy to carry out. The biofilm is analysed using plate counts on nutrient agar or R_2A agar. The nutrient agar is a nutrient-rich medium that allows most organisms to grow, while the R_2A medium is more specifically designed for water organisms and contain a lower level of nutrients (Vrouwenvelder et al. 2008; Dreszer et al. 2013; Reasoner & Geldreich 1985). EPS (usually measuring the proteins and

polysaccharide), ATP, TBC (staining/epi-fluorescence are used in some cases) and TOC analysis are also carried out (Veza et al. 2008).

In many cases, *in situ* analysis of the biofilm is not possible when carrying out membrane autopsies. The biofilm must first be removed from the membrane surface before it can be analysed. Many different techniques are used to remove the biofilm from the membrane. Suwarno et al. (2012) made use of a sonicator and subsequently agitated at high speeds using a vortex mixer to detach the biofilm from the membrane surface. The EPS and CFUs were then determined from the biofilm suspension without any treatment of the removed biofilm (Suwarno et al. 2012). Vrouwenvelder et al. (2008) also made use of 2-min ultrasonic treatment, followed by vortex mixing, to remove the biofilm from industrial membranes. This treatment method was repeated three times before further analysis of the sample. This step was repeated three times (Vrouwenvelder et al. 2008). Dreszer et al. (2013) used a cell scraper to remove the biofilm from the membranes, shaken for 30 minutes and then treated in an ultrasonic bath for two minutes, followed by ten pulses from an ultrasonic probe for homogenisation. The EPS was then isolated using cation exchange resin to dissolved the EPS. The cation exchange resin removed cations from the EPS, leading to the breakup of the EPS matrix. The sample was then centrifuged to separate the cells from the supernatant (Frolund et al. 1996; Dreszer et al. 2013). It is not obvious which of these techniques are the most effective to remove the biofilm from the membranes, because different feed water sources were used in these studies and different tests were carried out on the removed biofilm. Therefore is necessary to do a more detailed comparison of the different techniques to identify and select the best method.

The EPS can also be extracted from the EPS/cell suspension to study the physiochemical properties of the EPS better. The EPS can be extracted once the biofilm has been removed from membrane. A number of methods can be used to extract the EPS. They can be divided into chemical and physical methods. Chemical methods include the use of EDTA, NaOH and formaldehyde, while physical methods include heating, sonication and high-speed centrifugation. The chemicals used for EPS, extraction have different working mechanisms, e.g. the EDTA increases the solubility of the EPS while the pH change as a result of the NaOH resulted in separation between the acidic groups in the EPS (Pan et al. 2010). Extraction with chemicals gives higher EPS yields than the physical methods. Pan et al. (2010) found that extraction with EDTA or NAOH and formaldehyde increased the yield by about one order of magnitude compared to centrifugation and ultrasound. It is however shown that chemical methods alter the composition of the EPS (Pan et al. 2010; Liu & Fang 2002; Comte et al. 2007). Chemical methods may therefore be undesirable to separate the EPS from the cell suspension. It is therefore recommended EPS extraction should only be used if specific information of the EPS is required and not when only the amount of EPS present must be determined.

2.4 Prevention of biofouling

Membrane fouling causes a reduction in plant capacity. It is necessary to prevent and control biofouling in order to prevent a decrease in plant throughput. It is also necessary to prevent and control biofouling when the plant is offline for maintenance and not operational for extended periods. Biofouling can further become a problem when newly manufactured membranes are shipped to their intended location and must therefore be controlled (Al-Juboori & Yusaf 2012).

There are two approaches followed to overcome the problem of biofouling. The first approach is the prevention of biofouling by adequate pretreatment of the feed to the membranes. The second is when biofouling has already occurred. Chemical cleaning is then used to restore the membrane to the original conditions (Al-Juboori & Yusaf 2012).

2.4.1 Feed pretreatment

A reduction in fouling potential of the feed water is required before the feed enters the RO plant. This is done during the pretreatment process. Conventional pretreatment includes coagulation/flocculation, dissolved air flotation or settling, activated carbon adsorption and media filtration, while membrane processes include microfiltration, ultra-filtration and nano-filtration. The pretreatment process removes particulates, microorganisms, micro-pollutants and helps with the prevention of scaling by adding antiscalants. Membrane pretreatment processes are more effective in bacterial removal, but have a higher capital and operating cost (Kim et al. 2009). Shock dosing with chlorine, followed by dechlorination with sodium metabisulphite, is often used to disinfect the feed lines and pre-filter systems (The Dow Chemical Company 2015).

(a) Nutrient removal

Bacteria use a wide range of dissolved organic carbon for metabolic activities, of which low molecular weight molecules are utilised more (Amon & Benner 1996). By limiting the amount of nutrients fed to the bacteria, the growth can be controlled better. Only the removal of nutrients is targeted, and not the prevention of other types of fouling, which include scaling, organic adhesion and particle deposition. The previously mentioned AOC is considered as one of the most important factors in controlling the heterotrophic bacteria activity and attached biomass, since AOC can easily be assimilated by the microorganisms (Van der Kooij 1992).

Dissolved organic carbon can be removed by different treatment processes, depending on the desired concentration. Different levels have been reported to limit microbial growth in unchlorinated water. LeChevallier et al. (1992) report a value of less than 100 µg/ℓ; Brandford et al. (1994) report a level of 50 µg/ℓ or less and Van der Kooij (1992) reports a value of less than 10 µg/ℓ. Conventional treatment can reduce the AOC concentrations to around 50 µg/ℓ. The AOC concentration can be

further reduced by activated carbon adsorption, slow sand filtration, or membrane filtration (Nguyen et al. 2012). A biofilter often develops when an activated carbon filter is used over time. Active biomass then grows inside the filter, leading to a reduction in AOC (Hu et al. 2005).

The removal of phosphate is also known to reduce biofouling of membrane surfaces. The use of certain antiscalants (phosphate-based antiscalants) can increase the phosphate concentration, increasing the risk of biofouling (Vrouwenvelder et al. 2010). It is recommended that phosphate-free antiscalants should be used to reduce the potential for biofouling.

2.4.2 Surface modification

The surface of the membrane plays an important role in the attachment of the bacteria. The adhesion of bacteria is less with a hydrophilic membrane, and when a smooth membrane is used (Kochkodan et al. 2006). Lee et al. (2010), however, have found that the surface properties of the membrane (hydrophobicity or hydrophilicity) only had an influence on the initial attachment of microorganisms on the membrane. They (Lee et al. 2010) have also found that the growth became similar after 48 h, regardless of the different surface properties. Baek et al. (2011) report that the surface properties (hydrophobicity, roughness and surface charge) of commercially available RO membranes have a limited influence on biofouling measured with the flux decline over time.

The use of nanomaterials has become popular with the growth of the nano-industry. Nanomaterials are active on the membrane surface and do not only act as a physical barrier for microorganisms to attach to the surface. It has been shown that different nanomaterials have strong antimicrobial properties. These nanomaterials include silver nanoparticles, chitosan, photocatalytic TiO_2, carbon nanotubes, zeolite, graphene oxides and aqueous fullerene (nC60) nanoparticles (Matin et al. 2011; Ong et al. 2016).

2.4.3 Chemical techniques

A wide variety of chemical reagents can be used to control and overcome biological fouling on membranes and reduce the number of viable cells in the feed water to the membranes. These chemicals can be divided into two classes: oxidising and non-oxidising biocides. Oxidising biocides include chlorine, ozone, chloramines and chlorine dioxide. Non-oxidising biocides include 2,2-dibromo-3-nitrilopropionamide (DBNPA), sodium bisulphite and isothiazolone (Kucera 2010; Al-Juboori & Yusaf 2012).

Oxidising biocides (especially chlorine) have the ability to oxidise polyamide composite membranes, causing a rapid decline in salt rejection. A chlorine exposure of 200-1000 ppm hours of free-chlorine exposure can lead to a decline in salt rejection. Cellulose acetate membranes however, can tolerate a

continuous chlorine exposure of 1 ppm. The oxidation reaction will be catalysed in the presence of transitional metals such as iron. A high pH increases the reaction rate. Carbon filtration, sodium metabisulphite and UV radiation can be used to remove free chlorine from the feed water prior to the RO plant (Kucera 2010).

(a) Non-oxidising biocides

DBNPA: Also known as 2,2-dibromo-2-cyanoacetamide or 2,2-dibromo-2-carbamoylacetonitrile is the active ingredient in numerous available biocides used for water treatment. DBNPA is a white powder with a water solubility of 15 g/l at 20 ^{o}C (Wolf & Sterner 1972).

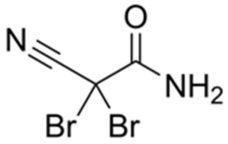


Figure 2-12: Chemical structure of DBNPA

DBNPA is easily hydrolysed in water with a half-life of a few hours, which decreases with an increase in solution pH. DBNPA has a half-life of 155 hours at a pH of 6 and 25 ^{o}C. This decreases to 2 hours at pH 8 and 25 ^{o}C (Exner et al. 1973). A stable solution is formed when DBNPA is dissolved in an acidic solution. It is especially soluble in polyethylene glycol, making it a preferred solvent.

DBNPA is a powerful, fast-acting biocide, which kills organisms upon contact. The antimicrobial activity of DBNPA is fast enough before hydrolysis of the chemical can occur. Relative nontoxic products are formed by the degradation of DBNPA through two pathways. The presence of organic materials causes degradation with a second pathway. The ultimate degradation will form ammonia, carbon dioxide and bromide ion (Blanchard et al. 1987).

DBNPA can be used on a continuous or shock-feed basis. A wide variety of dosages, dosing durations and dosing intervals is recommended. This will depend on the required permeate quality, type of water system and microbial fouling potential of the water (Kucera 2010; Dow Chemical Company 2000). DBNPA should also not be used with sodium bisulphite, since it will reduce the active ingredient in DBNPA (Kucera 2010). Shock treatment is typically used when ultra-pure water or potable water is required, since not all the degraded products are always rejected by the membranes.

DBNPA rapidly inhibits the growth and metabolism of the cells, followed by irreversible cell damage, resulting in the loss of viability. The biocide functions as an electrophilic agent, inhibiting growth by reacting with critical enzymes (Williams 2007).

Sodium Bisulphite: Sodium bisulphite (SBS) binds oxygen, making the oxygen unavailable for microorganism consumption. The efficiency of SBS is also influenced by the type of microorganisms

encountered, with certain microorganisms such as the anaerobic bacteria showing resistance to SBS (Nguyen et al. 2012).

Sodium bisulphite can be used continuously, or on a shock-feed basis. The concentration will mostly depend on the temperature of the water and the nutrients available in the water, since these two parameters have a significant influence on the growth of microorganisms (Applegate & Erkenbrecher 1987). Typical dosing concentrations range between 500 and 1000 ppm for 30 minutes or longer.

Isothiazolone: Isothiazolones rapidly inhibit cell growth and metabolic activities, followed by irreversible cell damage. The required contact time is, however, much longer compared to DBNPA. A dosage of 50 to 100 ppm requires a residence time of 4 hours. Given the long contact time of isothiazolone, it is recommended that it is used during cleaning events and not during slug/continuous dosing. Although microorganisms can become resistance to isothiazolones, it is more effective than DBNPA in water with a high organic concentration (Kucera 2010; Williams 2007).

Glutaraldenhyde: Glutaraldenhyde chemically modifies or cross-links amines (commonly found on proteins and microbial cells), thereby inactivating the proteins and cells. This then results in cell death. Typical dosing concentrations range between 20 and 100 ppm (McGinley 2012).

(b) Oxidising biocides

Chlorination: Sodium hypochlorite (NaOCl) or chlorine gas (Cl_2) is added during chlorination. When added to water, it immediately hydrolyses to form HOCl and hydrochloric acids. The reactions are shown below.

$$Cl_2 + H_2O \leftrightarrow HOCl + HCl \qquad \textbf{Equation 2-19}$$

$$HOCl \leftrightarrow OCl^- + H^+ \qquad \textbf{Equation 2-20}$$

Hypochlorous acid (HOCl) is unstable in water and will decompose, according to *Equation 2-20*. HOCl and OCl^- are strong oxidative agents, but OCl^- is considered weaker, because it cannot penetrate negatively charged cells.

A major advantage of the use of chlorination is residual effect left in the water, preventing regrowth of microorganisms. It is very effective against viruses and bacteria, with poor to moderate effect against endospores and protozoa. However, it cannot be used directly on polyamide membranes, because the strong oxidising power of chlorine causes the oxidation of membranes. It can also cause the formation of carcinogenic disinfection by-products, which can again be reduced by the use of chloramines. (Al-Juboori & Yusaf 2012)

Ozone: Ozone is a very powerful oxidising agent, more so than chlorine, that cannot be used directly on polyamide membranes. Hydroxyl (OH^-) free radicals are formed during the decomposition of

ozone in water. These radicals bind to each other to form peroxide (H_2O_2) that are effective against microorganisms. Ozone has the ability to deactivate bacteria, viruses, endospores and protozoa. It can also oxidise the organic components in the water. (Kucera 2010). This may lead to an increase in the dissolved carbon in the water, providing more nutrients for the microorganisms (Valavala et al. 2011). However, ozone is expensive to generate and possible side reactions with ozone can lead to carcinogenic components (Kucera 2010; Al-Juboori & Yusaf 2012).

2.4.4 Biochemical techniques

Enzymes can be used to weaken the EPS matrix that surrounds the cells on the membrane surface. Biochemical agents include enzymes, bacteriophages and signalling molecules. This is done by shortening the chain length of the polymers inside the EPS (Flemming et al. 2011). A wide variety of enzymes exists that can degrade the EPS. These enzymes can be grouped into two classes: polysaccharide lyases and hydrolases (Al-Juboori & Yusaf 2012).

Bacteriophages are viruses that can multiply rapidly within bacteria (Goldman et al. 2009). Bacteriophages consist of a wide variety of enzymes that have the ability to degrade polysaccharides. A bacteriophage can only affect a limited number of polysaccharide structures, making it less ideal for biofilm control. Due to the wide variety of different microorganisms and structures on a bio-fouled membrane surface, a single phage or phage combination is not sufficient to remove the biofilm (Flemming et al. 2011).

Another approach is the use of signalling molecules to remove biofilms, although not successful in technical systems to date. Signal molecules are responsible for malignancy and the release of certain chemicals that degrade polysaccharides in bacteria. The bulk use of signalling molecules is very costly and is limited to bacteria in their vegetative states. Vegetative-state bacteria are only a very small portion of the biofouling on the membrane, which limits the use of signalling molecules (Flemming et al. 2011).

2.4.5 Other techniques

There are still many different strategies available for the control and prevention of biofouling. Some of these strategies include thermal disinfection, electrical, ultraviolet light (UV), ultrasound, pH control and gas flushing.

Thermal disinfection or solar disinfection is the disinfection of water using heat. A relatively high temperature (60-100 ^{o}C) is required to kill vegetative bacteria (Zhang et al. 2007). This will result in high cost. Solar disinfection uses a combination of heat and ultraviolet radiation to deactivate the bacteria. Electrical disinfection techniques consist of electro-chemical techniques and Pulsed Electric

Field (PEF). The electro-chemical technique makes use of direct electrolyses and mixed oxidant generators. The PEF, on the other hand, makes use of a high electrical field for a short time between two electrodes, which then affects the DNA and RNA of the organisms (Al-Juboori & Yusaf 2012).

UV irradiation can be used to deactivate chemical resistant bacteria. UV irradiation also breaks down organic components in the water, creating more consumable carbon for the microorganisms (Choi & Choi 2010). Deactivation through UV light is done by altering the DNA structure of the organisms (mutations), taking away their ability to reproduce (Kucera 2010). An alternative to UV irradiation and chlorination is ultrasound. Ultrasound creates acoustic cavitations in the water. The microorganisms are disrupted by these cavitations, because they have a mechanical influence on the cells, and create localised hot spots and free radicals that disrupt the cells (Al-Juboori & Yusaf 2012).

It has also already been mentioned that the pH of the solution has an influence on the adhesion of microorganisms to the membrane surface. The pH of a feed can be adjusted using a strong acid (HCl) or base (NaOH) (Al-Juboori & Yusaf 2012).

Chemical cleaning does not necessarily remove the biofilm from the membrane or from the feed space. Flushing of the membrane is therefore necessary to remove the biofilm. Water or a water/gas combination can be used to flush the membrane and remove the fouling. According to Ngene et al. (2010), water is saturated the most effectively with CO_2. Depressurisation causes bubbles to nucleate in the feed spacer, increasing cleaning efficiency (Ngene et al. 2010).

2.5 Fouling and flux loss allowed before cleaning

The RO membranes will eventually foul, regardless of the pretreatment process or hydraulic design of the membrane system. Cleaning of the membrane system must therefore be carried out to restore the performance of the system. This is known as cleaning in place (CIP). Sufficient and effective cleaning is necessary to keep the membranes foulant free. This will in turn save costs, extend membrane life and reduce the impact on the environment.

As mentioned before (Section 2.1.9), membranes are fouled by a number of different components. These foulants will cause a drop in normalised pressure, normalised flow and normalised salt passage. The system should therefore be cleaned when one or more of the following parameters are applicable (Kucera 2010):

- 10% decrease in normalised permeate flow
- 10-15% increase in normalised pressure drop
- 5-10% increase in salt passage

If cleaning is not done in time, foulants can become permanently attached to the membrane or membrane components. The cleaning intervals also become more frequent and elements will foul or scale quicker. Cleaning is usually scheduled when the performance decrease by 10% and should be completed by the time the performance has changed by 15%.

Cleaning is usually not performed when there is an increase in salt passage. This is because the increase in salt passage can often be contributed to mechanical causes. The differential pressure over the feed channel in the different elements should also be monitored. Excessive increase in pressure will cause telescoping.

2.6 Summary

The most important points from the literature study are summarised below:

- Reliable prevention and control of biofouling on a polyamide RO membrane is difficult. While a wide variety of non-oxidising biocide dosages, dosing durations and frequencies are recommended in literature, there is still much controversy, making it difficult to select the best dosing strategy. Therefore, further investigations can certainly assist in the identification of optimal dosing strategies for improved microbial control.
- A very wide variety of microorganisms grows on RO membrane surfaces. It is hardly possible to prepare a synthetic feed (containing known microorganisms) that will be representative of the microbial diversity on the membrane surfaces. Natural feed water containing nutrients are able to stimulate the growth of a diverse microbial community.
- Removal of a biofilm from the membrane surface is critical in biofouling analysis. A wide variety of methods is currently used for biofilm removal and the homogenisation thereafter. These methods include ultrasonic bath treatment, scraping from the membrane surface and treatment with an ultrasonic probe or a combination of these methods. Unfortunately, the best combinations of these methods have not yet been determined, therefore posing notable uncertainty about the removal methodology that should be followed.
- Normalised flux, protein and polysaccharide concentration, CFU and total cell count are relatively easy to measure and provide valuable information about the formation of the biofouling. However, biofouling can sometimes be difficult to quantify because there is currently a limited number of biological parameters that can be linked directly to the amount of biofouling on the membrane. Establishing a possible link between the measured parameters (e.g. protein- and polysaccharide concentration, CFU and total cell count) and flux may help to quantify biofouling better.

CHAPTER 3: MATERIALS AND METHODS

3.1 Experimental setup

An experimental setup was constructed to evaluate the biofilm development on flat-sheet polyamide reverse-osmosis membranes. The setup consisted of four identical cross-flow units. The blocks were constructed from polyvinylchloride (PVC). Each unit was able to house a membrane with an effective area of around 120 cm^2. XLE-4040 membranes were used, supplied by DOW. The XLE-4040 is a polyamide thin-film composite, extra low energy membrane. The recommended applied pressure is 6.9 bar with a permeate flow rate of 9.8 m^3/d and a stabilized salt rejection of 99.0 % (Dow 2015). The height of the feed channel was 800 µm. This height was selected to house the XLE-4040 feed spacer (711µm thick) with a 90º string structure. 12 mm steel plates were also used to keep the PVC block from deforming under the pressure. A simplified representation of the blocks is shown in Figure 3-1. The detailed block design is presented in Appendix A.

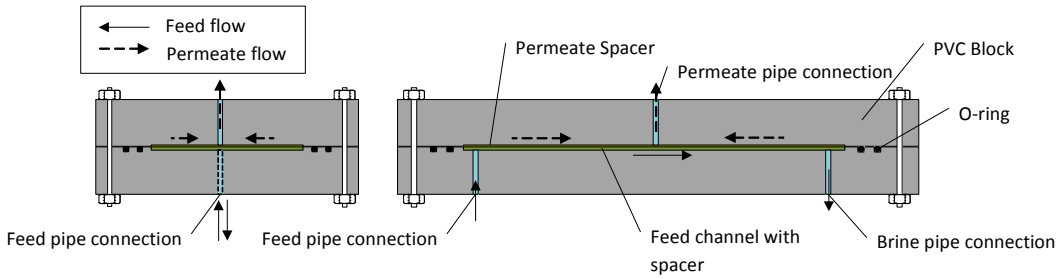


Figure 3-1: Simplified representation of the cross-flow filtration cell

The process flow diagram of the setup is shown in Figure 3-2. Tap water supplied by the Stellenbosch Municipality was filtered through granular-activated carbon (GAC), followed by a 1 µm pore-size cartridge filter. The GAC removed any residual free chlorine from the water. The GAC also removed organic components. The cartridge filter removed particulates above 1 micron.

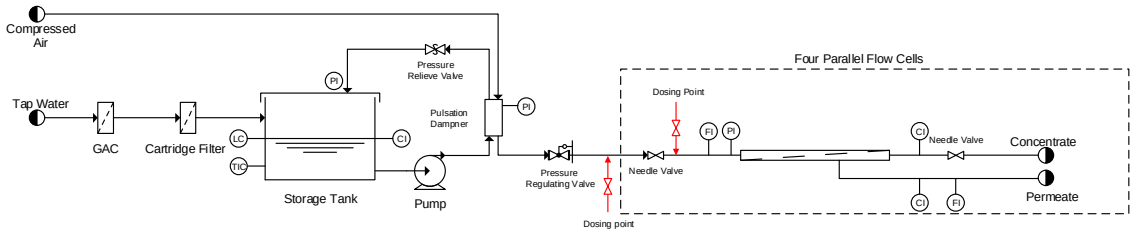


Figure 3-2: Process flow diagram of experimental setup

The temperature was controlled in a holding tank on the suction side of the feed pump (Sera RF 409.1-18). A pressure-relieve valve (½" Comet type) was used to ensure that the pressure in the self-

constructed pulsation damper did not exceed the desired pressure. Compressed air was also supplied to the pulsation damper to ensure the damper performed satisfactory.

A pressure-regulating valve (Parker Pneumatic, 20R113GC) was used to control the pressure to the four identical blocks shown in Figure 3-2. The feed pressure was varied between 3-bar and 7-bar gauge. Flow to all the blocks was monitored with rotameters (Omega, FL-3803ST-NY). The permeate flux was measured with a beaker and stopwatch.

3.2 Reagents

The chemicals and their attributes used in this study are listed in Table 3-1.

Table 3-1: List of chemical reagents used and their attributes

Component	Composition	Assay	Supplier
DBNPA	$C_3H_2Br_2N2O$	≥99.0%	Classic Chemicals
Glucose	$C_6H_{12}O_6$	≥99.5%	Sigma Life Science
Hydrochloric Acid	HCl	31.5-33.0%	BDH
Nutrient Agar	1 g meat extract, 5 g peptone, 2 g yeast extract, 8 g sodium chloride, 15 g agar per litre of water	-	Merck
Phenol	C_6H_6O	≥99.5%	Merck
Potassium Chloride	KCl	≥99.0%	Merck
Potassium Dihydrogen Orthophosphate	KH_2PO_4	≥99%	Merck
R_2A Agar	0.5 g yeast extract, 0.5 g Proteose Peptone hydrolysate, 0.5 g Casamino Acids, 0.5 g of glucose, 0.5 g soluble starch, 0.3 g K_2HPO_4, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.3 g sodium pyruvate and 15 g agar per litre of water	-	Merck
Sodium Acetate	CH_3COONa	≥99%	Merck
Sodium Chloride	NaCl	≥99.0%	Merck
Sodium Dihydrogen Orthophosphate	NaH_2PO_4	≥98%	Merck
Sodium Hydroxide	NaOH	≥98%	Saarchem
Sodium Nitrate	$NaNO_3$	≥98.5%	Merck
Sulphuric Acid	H_2SO_4	95-99%	Saarchem

3.3 Experimental procedure

3.3.1 Membrane preparation

Before each experiment, the system was thoroughly cleaned to ensure that no microorganisms or organic matter was left behind from the previous run. The cleaning was based on the cleaning method proposed by Herzberg & Elimelech (2007). A 0.01M NaOH solution at a temperature of 35 ^{o}C was first circulated through the system for one hour, followed by a rinse with DI water. This was followed by the circulating of a bleach solution (active ingredient is 10% sodium hypochlorite) to disinfect the system. The membrane blocks were then opened and cleaned with ethanol (75%). After the cleaning, DI water was circulated through the system and thoroughly flushed before the new membranes were added (Herzberg & Elimelech 2007).

After cleaning, the new membranes and feed-spacer material were inserted into the blocks and the blocks were closed. This was followed by membrane compaction using DI water at a pressure of 7 bar. An initial flux decline was observed if membrane compaction was not done. A constant flux was reached overnight. After membrane compaction, the tap water was added to the system and rinsed for one hour to ensure all the DI water was out of the system. The first flux measurement was then taken afterwards (Herzberg & Elimelech 2007; Huertas et al. 2008).

3.3.2 Feed water and added nutrients

Stellenbosch drinking water was used as feed water. Most of Stellenbosch's drinking water is supplied by raw water from the Eerste Rivier in the Jonkershoek Valley treated by sand filtration and chlorination. Water is also supplied from the Theewaterskloof Dam. This water is usually treated by rapid filtration and chlorination. The influent water to the treatment plants has a low pH and is high in colour. This is typical of the Southern Cape mountain waters.

A solution of sodium acetate (CH_3COONa), sodium nitrate ($NaNO_3$) and sodium dihydrogen orthophosphate (NaH_2PO_4) was used as nutrient solution with a mass ratio for C:N:P of 100:20:10, respectively, to give a final concentration of 100 µg carbon/litre. This nutrient composition has been used in several previous studies on biofilms and biofouling (Vrouwenvelder et al. 2010; Vrouwenvelder et al. 2009; Dreszer et al. 2014). Slug dosing of nutrients was performed daily in the feed-water tank to give a final concentration of 100 µg/ℓ carbon concentration in the feed water. This concentration has also been used in previous studies (Bucs et al. 2014; Vrouwenvelder et al. 2010).

3.3.3 DBNPA dosing

DBNPA was dosed using a peristaltic pump. A range of different DBNPA dosages was tested. The dosage, dosing duration and dosing frequency were varied to determine the optimal dosing strategy.

According to the Dow Chemical Company (2000), using a shock dosage of 10-30 ppm for 30 min to 3 h every five days should be efficient for water less prone to biological fouling and 30 ppm for the full 3 h if the feed water is more prone to biofouling. According to Kucera (2010), a dosage of 100 ppm for 30 to 60 min every two days to once a week should be sufficient, depending on the degree of biofouling potential. The different runs carried out are shown in Figure 3-3. A control block (without any DBNPA dosing) was used during each run to account for any variation in the feed water.

A high cross-flow velocity (1.26 cm/s) and low cross-flow velocity (0.45 cm/s), together with DBNPA dosing, were also used to investigate the hydrodynamic influence on biofilm development.

Figure 3-3: Summary of experimental runs

Run	Feed water	Run Time (days)	Block	Dosing (dosage, duration, frequency)
1	DI water	7	1	None
			2	
			3	
			4	
2	Tap water	7	1	Continuous DBNPA
			2	
			3	
			4	
3	Tap water	7	1	200 ppm, 15 min daily
			2	200 ppm, 30 min daily
			3	100 ppm, 15 min daily
			4	Control
4	Tap water	7	1	10 ppm, 2 h daily
			2	100 ppm, 30 min daily
			3	10 ppm, 30 min daily
			4	Control
5	Tap water	7	1	10 ppm, 2 h daily
			2	Control
			3	100 ppm, 30 min, every 2nd day
			4	Dead End
6	Tap water	7	1	10 ppm, 2 h daily
			2	100 ppm, 30 min, daily
			3	10 ppm, 30 min, daily
			4	Control
7	Tap water + 100 µg/ℓ C	9	1	100 ppm, 30 min, daily
			2	100 ppm, 30 min, every 2nd day
			3	Control
			4	10 ppm, 30min daily

8	Tap water + 100 µg/ℓ C	9	1	100 ppm, 2 h, daily
			2	100 ppm, 2 h, every 2nd day
			3	Control
			4	10 ppm, 2 h, daily
9	Tap water + 100 µg/ℓ C	9	1	10 ppm, 2 h, every 2nd day
			2	10 ppm, 30 min, every 2nd day
			3	Control
			4	10 ppm, 30 min, daily
10	Tap water + 100 µg/ℓ C	11	1	200 ppm, 30 min, daily
			2	100 ppm, 30 min, 2 x per day
			3	Control
			4	100 ppm, 1 h, daily

3.4 Biofilm analysis

The blocks containing the membranes were opened when the defined operating time was reached. As discussed in the Section 2.3.3, different methods were used for the removal of the biofilm from the membrane and the homogenisation of the biofilm. A combination of different methods was therefore used to determine the best approach to remove the biofilm. The removal consists of different combinations of methods, which include scraping the biofilm from the membrane using a sterile spatula, treatment in an ultrasonic bath and treatment with an ultrasonic probe (Hielscher, UP400S). The output for the ultrasonic probe was measured with a VOLCRAFT® energy check 3000 (Conrad, Ettlingen, Germany). The measured output was 32.12±2.28W, which was shown to be low enough not to cause significant cell lysis (Dreszer et al. 2013).

The following techniques were used to remove the biofilm from the membrane:

- Scraping the biofilm from the membrane (using the sharp end of a spatula).
- Scraping the biofilm from the membrane and treating the membrane in an ultrasonic bath for 1 min.

The homogenisation was done as follows:

- Treatment in an ultrasonic bath for 2 min.
- Treatment with an ultrasonic probe in pulsating mode for 10 pulses at 45%.
- A combination of ultrasonic bath and ultrasonic probe.

With all the methods used, the biofilm was suspended in phosphate buffered saline (PBS: NaCl 8 g/l, KCl 0.2 g/l, $Na_2HPO_4{\cdot}7H_2O$ 1.15gg/l and KH_2PO_4 0.2 g/l; the pH was adjusted to 7.3 using either HCl or NaOH) solution. The solution was gently vortexed between each step of the removal process. The full flow diagram of the different combinations used can be seen in Figure 4-2.

The difference between the biofilm removal techniques is that, in the one case, the membrane is discarded. This technique had been used by Dreszer et al. (2013), although it can lead to biofilm losses. The biofilm removed from the membrane consists of clumps of cells interspersed with EPS material. Homogenisation was found to be effective to liberate the cells from the EPS. However, whereas excessively harsh treatment could cause cell lysis, overly mild treatment could prevent effective dissolution of EPS and cells, and breaking up of cell clumps.

Heterotrophic plate counts were then performed at the end of each step on NA and R_2A agar. Plate-count procedures are discussed in more detail in Section 3.5.4. The method with the highest plate counts indicates that more cells have been liberated without cell lysis, since the membranes used were fouled under the same conditions during the same run.

The feed spacer was only treated using the ultrasonic bath. It was not possible to scrape cells from the spacer material. Large cell clusters were also not observed when the biofilm was removed from the feed spacers.

3.5 Data collection

3.5.1 Flux decline

The feed pressure was kept constant for all the membrane blocks during a run. The permeate flux through the different membrane elements was then measured to monitor the flux decline. The temperature and conductivity were also measured (with YSI, Model 63 handheld meter) to be used for data normalisation. This is discussed in more detail in Section 2.1.7.

The flow was then normalised using the "FTNORM" software from *Dow*®. The calculation method used by the software is discussed in Section 2.1.7. The software normalises the data to account for the variations in temperature, feed pressure and feed-channel pressure drop and the osmotic pressures of the feed and permeate. It was assumed that the pressure on the permeate side was atmospheric pressure, since the pipe connecting it to the atmosphere was assumed to have negligible pressure drop. The pressure drop over the feed channel was also assumed negligible compared to the feed pressure. The blocks used by Bucs et al. (2014) to house the membrane sheets were similar in size, had similar cross-flow velocities and gave a pressure drop of around 100 Pa without a large increase over the course of the experiment.

3.5.2 Protein

The amount of protein in the EPS was analysed using the bicinchoninic acid assay (BCA) kit (Novagen®, Damstadt, Germany). A sample or standard of 50 µl was added to 1 ml BCA working

reagent and gently vortexed. BCA working agent was prepared using 50 parts BCA solution with one part of 4% Cupric Sulphate. The samples were then incubated in a water bath at 37 ^{o}C for 30 min and allowed to cool to room temperature. The reactions were then transferred to clean cuvettes and the absorbance was measured at 562 nm (Varian, Cary 1E UV-VIS Spectrophotometer, SMM Instruments). All absorbance measurements were taken within 10 min of each other, since the reaction is continuous at room temperature, even if slow. Bovine serum albumin (BSA) was used as standard. The standard curve can be seen in Appendix C.

In brief, the BCA assay, consisting of bicinchoninic acid and sodium salt, is capable of forming a purple complex with the cuprous ion (Cu^{+1}) by the protein reduction of Cu^{+2} to Cu^{+1} (biuret reaction) in an alkaline environment. A proportional increase in colour is produced with an increase in protein concentration (Smith et al. 1985).

3.5.3 Polysaccharides

The amount of polysaccharides in the EPS was measured using the phenol-sulphuric acid method. 200 µl of 5% (w/v) phenol solution and 1 ml of concentrated sulphuric acid (H_2SO_4) were added to 400 µl samples. The solution was then mixed and allowed to cool down. The reactions were then transferred to clean cuvettes and the absorbance measurements taken at 490 nm (Cary UV-Visible Spectrophotometer). Glucose was used as polysaccharide standard. The standard curve can be seen in Appendix C.

In brief, oligosaccharides, simple sugars, polysaccharides and their derivatives with a free or potentially free reducing group gives of a stable orange colour when treated with phenol and concentrated sulphuric acid, resulting in a linear relationship between reducing sugar concentration and the colour reaction, measured as absorbance, within a predetermined range before deviations from linearity occur. This technique is sensitive and can measure a sugar content of less than a microgram per litre (Dubois et al. 1956).

3.5.4 Plate counts

Colony-forming units (CFUs) were performed on nutrient agar (NA) and R_2A agar (from Merck Millipore). 100 µl of serially diluted cells and EPS suspension in PBS were spread onto the agar plates using an L-formed glass spreader. The NA agar plates were incubated for 48 h, counted and incubated again until five days before it was counted again. The R_2A agar plates were incubated for five days only. The incubation was done at 35 ^{o}C. These times and temperatures were recommended by the manufactures. Triplicates of each dilution series were counted and the mean reported. Plates with colony counts between 25 and 250 were counted. The average of the plates (taking the dilution

factor into account) was taken if two dilutions with countable colonies were encountered (Scott 2011). Both mediums were prepared by dissolving the agar in DI water and autoclaving at 121 ^{o}C for 15 min.

NA is a rich culture medium for the cultivation of less fastidious microorganisms. R_2A agar is a medium that contains a low nutrient content, especially suited for water organisms. Both these media were evaluated.

3.5.5 Cell counts

Total cell number (TCN) counts were performed using a Petroff Hausser counting chamber (Naubauer improved, Marienfeld, Germany) with a depth of 0.01 mm and a light microscope (supplied by Lasec SA). Triplicate counts of twenty squares each were counted for a dilution series for the biofilm. This was only done once the biofilm removal and homogenisation technique were determined. The averages of the triplicate counts were then taken. A single count of twenty squares for the feed spaces was counted. The total number of cells was determined with the following equation:

$$TCN = \frac{Cells\ counted}{counted\ area\ (mm^2) \cdot chamber\ depth\ (mm) \cdot dilution}\ [cells/\mu l] \qquad \textbf{Equation 3-1}$$

Random squares were counted. When cells touched the lines, only the cells touching the left and upper lines were counted. An illustration of this can be seen in Figure 3-4. Figure 3-4 (b), square 7 indicates the cells that were counted. Since the cell clusters observed were counted as one cell, this approach could have caused an underestimation of the total cell count. However, since cell clusters were observed in only a small number of the total bocks counted, blocks were predominantly selected displaying single cells. Whereas this approach could constitute a measure of non-sampling error, it was reasoned that counting a sufficiently large number of blocks (20 blocks per count estimate) and then also performing counts in triplicate, would minimise potential estimation errors.

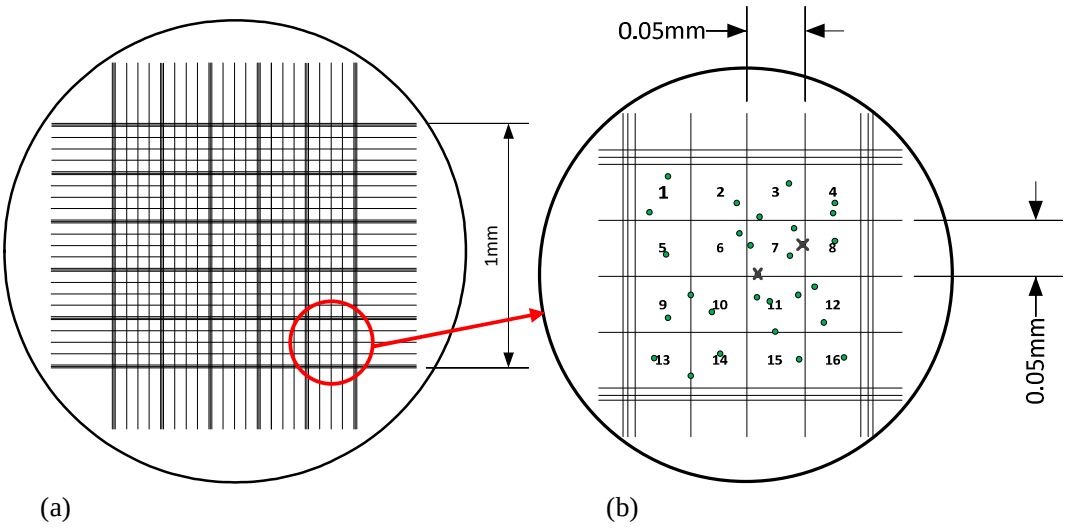


Figure 3-4: Petroff Hausser counting chamber grid at different magnifications

3.5.6 Gram staining

The gram-stain technique is used to differentiate between two classes of bacteria, namely gram-positive and gram-negative bacteria. In the first step, the smear is stained with basic crystal-violet dye. This primary stain allows intercalation of the dye molecules and the peptidoglycan of the bacterial cell wall. An iodine solution is then used as a mordant by making the peptidoglycan more rigid, so that the cells are stained more strongly. The smear is then decolourised by washing with ethanol or acetone. The ethanol/acetone will wash the dye from gram-negative cells due to a much thinner peptidoglycan layer than the gram-positive bacteria, which will retain the crystal violet. Safranin (a common counter stain) used to stain the gram-negative bacteria pink/red and left the gram-positive bacteria dark purple.

The difference in cell walls between gram-positive and gram-negative cells is responsible for the different colour changes. Gram-positive cells have thick cell walls that are primarily composed of peptidoglycan. Gram-negative cells have much more complex cell walls. Only a thin layer of peptidoglycan is present in the cell wall. The peptidoglycan constitutes not more that 5-10% of the wall for gram-negative cells while the peptidoglycan constitutes between 50-90% of the cell wall for gram-positive bacteria. Due to the thinner layer of peptidoglycan in gram-negative cells, they are not able to retain the purple stain (Willley et al. 2008).

The steps followed for gram staining were as follows (Rollins 2000):

1. A loopful of the dissolved biofilm is transferred to the surface of a clean glass slide and spread over an area.
2. The glass slide is then passed over a Bunsen flame to fixate the cells to the slide. The cells are also not directly exposed to the flame.

3. The slide is then flooded with crystal violet for one minute and then rinsed off with water and drained.
4. Flood the slide with Gram's iodine solution for one minute and rinse off with water and drain.
5. Remove excess water from slide and add 95% ethanol to the slide for 10 seconds. Drain the slide afterwards.
6. Flood the slide with safranin solution for 30 seconds. Rinse off and drain slide.
7. The slide can be blot dried with bibulous paper, but do not rub.
8. The slide can then be examined under an oil-immersion lens at 1000-fold magnification.

3.6 Statistical analysis

Statistical analysis was carried out using the data analysis tool in Excel 2007 and Statistica (Statsoft) Statistical significance of 95% was considered as significant.

CHAPTER 4: RESULTS AND DISCUSSION

The growth of a biofilm under different conditions (supplementary nutrients and under different hydrodynamic conditions) was evaluated on bench-scale experimental equipment. This was accompanied by the evaluation of different biofilm removal and homogenization techniques currently used for biofilm removal. The effect of DBNPA dosing strategies (dosage, dosing duration and frequency) was investigated to determine an optimal dosing strategy. The results obtained from these experiments were then also used to identify biological parameters (protein concentration, polysaccharide concentration, total cell count and CFU) that could be used to quantify biofouling by linking it to a typical system parameter (flux decline).

4.1 Development/evaluation of biofilm removal method

4.1.1 Characterisation of microbial-EPS clumps using light microscopy

Several different methods were used to remove the biofilm from the membrane surface, as discussed in Section 2.3.3. The most common methods rely on a combination of scraping and ultrasound treatment to remove the biofilm from the membrane surface, followed by additional ultrasonic treatment to homogenise the cell suspension with high-speed mixing using a vortex between the different steps.

There were, however, still cell clusters that could be observed when the biofilm was removed from the membrane. The objective of the removal process was therefore to minimise the size and amount of these clusters, liberating as many cells from the EPS matrix as possible.

Figure 4-1 (A) shows the macroscopic cell clusters that were observed when the biofilm was scraped from the membrane and mixed using a vortex without nutrient dosing. Figure 4-1 (B) and (C) show the remaining clusters after the scraped biofilm was treated in an ultrasonic bath for 2 min and then with an ultrasonic probe for 10 pulses.

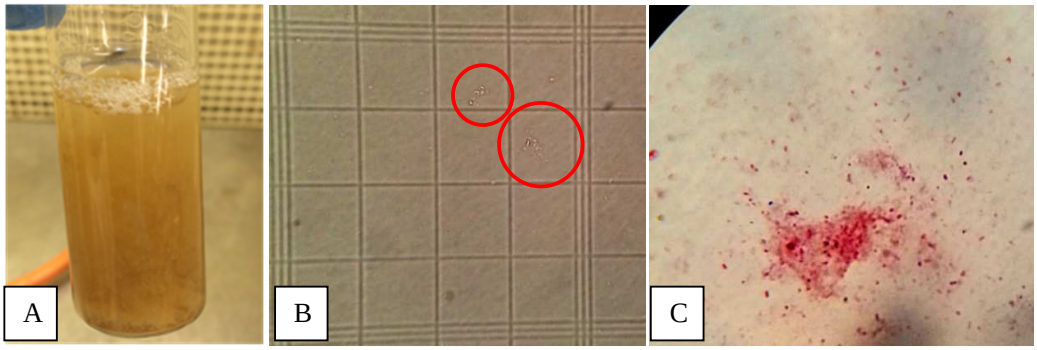


Figure 4-1: (A)-Macroscopic observation of removed biofilm suspended in PBS before homogenisation, (B)-Microscopic observation of cell clusters at 400-fold magnification in a counting chamber, (C)-Microscopic observation of cell clusters with gram staining of cells fixed to the microscope slide at 1 000-fold magnification

Gram staining was done to visually quantify the cells inside the cell clusters. Although, most of the cells were gram negative, the aim of the staining was not to determine the gram status, but rather provide a visualisation of the clumps, since it was not always very clear when using bright field microscopy.

4.1.2 Determination of preferred cultivation medium and biofilm removal technique

Nutrient agar (NA) and R_2A agar were tested simultaneously with the different removal and homogenisation techniques. This was done by allowing the same microbial growth to take place on all the membranes by using the same feed and running the blocks in parallel. The membranes were then randomly divided so that each treatment process was done three times. The cell suspension with the EPS was then plated in duplicate on the NA and R_2A agar after the treatment processes so that the treatments could be compared. Different incubation times are also recommended by manufacturers for the NA and R_2A agar. The influence of incubation time is also compared. Since the same fouled membranes were used as base for the treatment, the higher colony count will indicate that more cells have been liberated without cell lysis.

4.1.3 Efficiency of biofilm removal and EPS disruption

The removal of the biofilm from the membrane itself can be divided into two main techniques. This is illustrated in Figure 4-2. The first method is to scrape the biofilm from the membrane and suspended it in PBS solution while discarding the membrane afterwards. This assumes that the amount of cells

remaining on the membrane is negligible. The second method is to also scrape the biofilm from the membrane, but not discard the membrane afterwards. The membrane is then cut into 2x3 cm pieces before being treated in an ultrasonic bath for 1 min.

The scraped biofilm is subjected to treatment in an ultrasonic bath for 2 min, or with an ultrasonic probe for 10 pulses at 45% or a combination of both. The biofilm suspension is added to the cell suspension in the case where the membrane itself is not discarded.

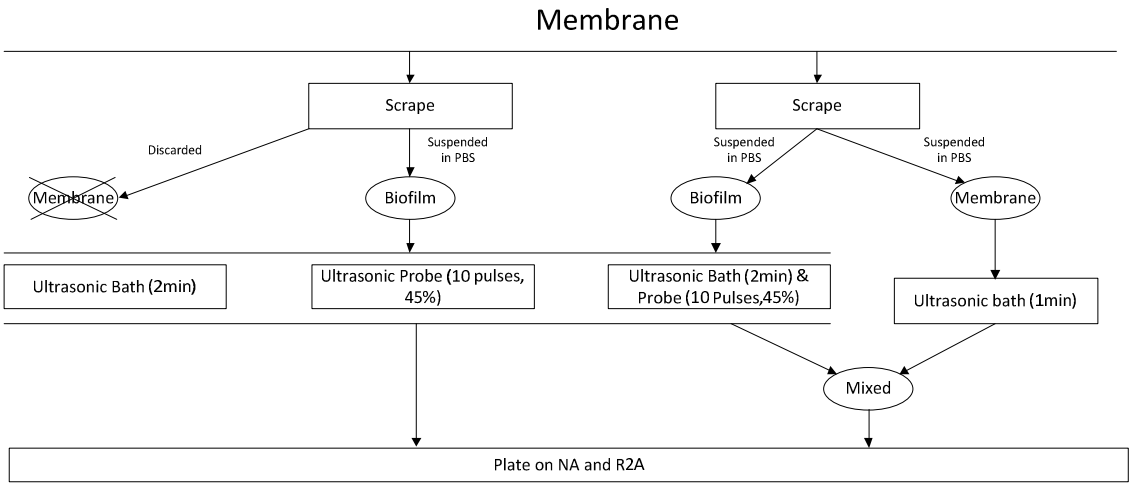


Figure 4-2: Flow diagram of biofilm removal from the membrane

The results are shown in Figure 4-3. The amount of microorganism growth is expressed as the number of colony forming units (CFU) per area of membrane that they were measured on. A membrane are of 60 cm^2 was used for each removal technique. The error bars is the standard error observed when the removal and homogenisation were carried out in triplicates.

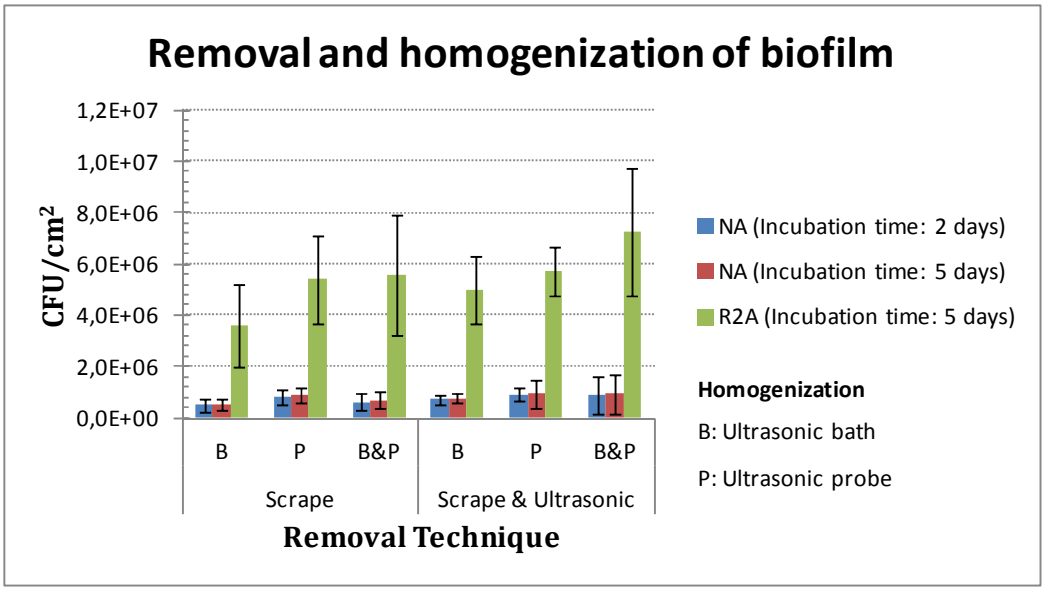


Figure 4-3: Removal and homogenisation of the biofilm using the different techniques measured on NA and R_2A agar. The error bars indicate the standard error observed when the experiment was carried out in triplicate

ANOVA analysis was carried out using agar type as the blocking factor, which allowed comparisons between agar types and within agar types. The R_2A agar clearly resulted in significantly greater CFU counts ($P << 0.05$) than when NA was used. However, the recommended incubation time for the R_2A agar was five days, compared to the two days for the NA. The longer incubation time can lead to an increase in CFU, since more time is allowed for bacterial growth. Nevertheless, no significant (p-value=0.46) increase in CFU/cm^2 was observed when the NA was incubated for an additional three days in order to have the same incubation time as the R_2A agar. Furthermore, within-group comparisons also showed significant differences (p=0.023) resulting from removal and homogenisation techniques, although not all removal techniques differed significantly. Lack of significance between some treatment techniques could in part be attributed to substantial variations when using plate counts.

It is clear that the R_2A agar gives much better results than the NA agar. The R_2A agar was therefore used throughout this study for the heterotrophic plate counts. It did therefore not really matter which removal technique was used. Suwarno et al. (2012) removed the cells from a single-strain model bacterium from the membrane using an ultrasonic bath for 1 min and mixing the suspension by using a votex. The CFU was also in the order of 10^6 CFU/cm^2. The treatment used by Dreszer et al. (2013) consisted of scraping the biofilm from the membrane, shaking it for 30 min, treatment in an ultrasonic bath for 2 min and then used an ultrasonic probe for 10 pulses. The yield varied between 10^8 and 10^9 CFU/cm^2. The higher yield can be contributed to a number of factors. Unchlorinated water was used as feed, which contained a higher concentration of microorganisms. The microorganisms were also not stressed due to the chlorination. The concentration of nutrients (1 mg/ℓ carbon) was also 10 times

higher than the nutrient concentration (100 µg/ℓ carbon) used further in this study. They used R_2A media but incubated at a temperature of 25 ^{o}C and for seven days, which can lead to higher bacteria counts as well (Dreszer et al. 2013). However, the data spreads of these two studies were similar to the spreads observed in this study.

Nevertheless, it was still decided to scrape the biofilm from the membrane and then submerge it in an ultrasonic bath, followed by treatment with an ultrasonic probe, because it gave the highest CFU/cm^2 count (although it was not significantly higher than the other techniques tested). This technique was used throughout this study.

4.2 Bio-fouling experiments without nutrient dosing

Membrane fouling tests were carried out to determine the capabilities of the experimental system and the extent to which biofouling could be achieved. No supplementary nutrients were added to stimulate biological growth on the membranes to determine if sufficient microbial growth could be obtained by only using tap water. The effect of hydrodynamics was also investigated. The experiments carried out during fouling runs are listed in Table 4–1.

Table 4-1: Biofouling runs without nutrients

Run	Feed water	Run time (days)	Block	Dosing (dosage, duration, frequency)
1	DI water	7	1	None
			2	
			3	
			4	
2	Tap water	7	1	Cont. DBNPA (7 ppm)
			2	
			3	
			4	
3	Tap water	7	1	200 ppm, 15 min, daily
			2	200 ppm, 30 min, daily
			3	100 ppm, 15 min, daily
			4	Control
4	Tap water	7	1	10 ppm, 2 h, daily
			2	100 ppm, 30 min, daily
			3	10 ppm, 30 min, daily
			4	Control
5	Tap water	7	1	10 ppm, 2 h, daily
			2	Control
			3	100 ppm, 30 min, every 2nd day
6	Tap water	7	1	10 ppm, 2 h daily
			2	100 ppm, 30 min, daily
			3	10 ppm, 30 min, daily
			4	Control

4.2.1 Test with demineralised water

A run with was carried out using demineralised water to determine if the membrane compaction was adequate and if a stable flux could be obtained for an extended period of time. The membranes were compacted at 7 bar overnight, after which the pressure was adjusted to 3 bar. The results are shown in Figure 4-4.

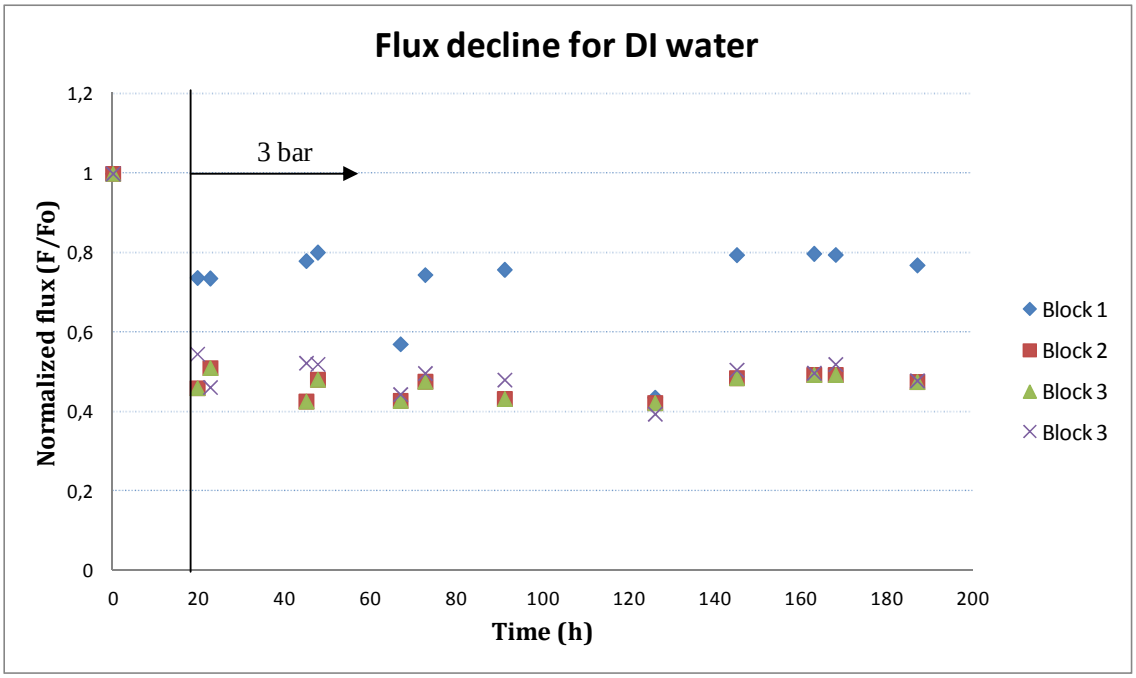


Figure 4-4: Normalised flux decline when DI water is used. The arrow indicates when the pressure was lowered from 7 bar to 3 bar. Blocks 1 to 4 were operated in parallel.

The initial flux was measured at 7 bar and used to normalise the data throughout the run. A relatively stable flux was observed, with a very stable flux after 140 h. Two definite outliers (at around 60 h and 120 h) are also observed for Block 1. A possible explanation for the outliers can be a result of the method used for the flux measurements. The permeate pipes were moved for the measurements. This could have caused some of the water in the pipe to spill, causing an air gap in the pipe, resulting in a measured flow rate that is lower than the actual flow rate. An initial error in the flux measurement of Block 1 can also be the cause of the higher normalised flux observed after compaction, although not confirmed.

A zigzag trend is observed in the data from 20 h to 80 h. Although the temperature of the feed water was regulated, large variations in the ambient temperature might have influenced the temperature of the block housing the membrane. The block was constructed from PVC and steel, making it difficult to heat. The ambient temperature, feed-water temperature and flux are compared in Figure 4-5. The daily ambient temperature measurements were made by the Sonbesie weather station located on the fifth floor of the civil engineering building at Stellenbosch University, which is close to the process engineering building where the tests were carried out. It should be noted that the daily temperature is only an indication of the ambient temperature in the building where the experiments were carried out, since the weather station is situated outside and on the fifth floor.

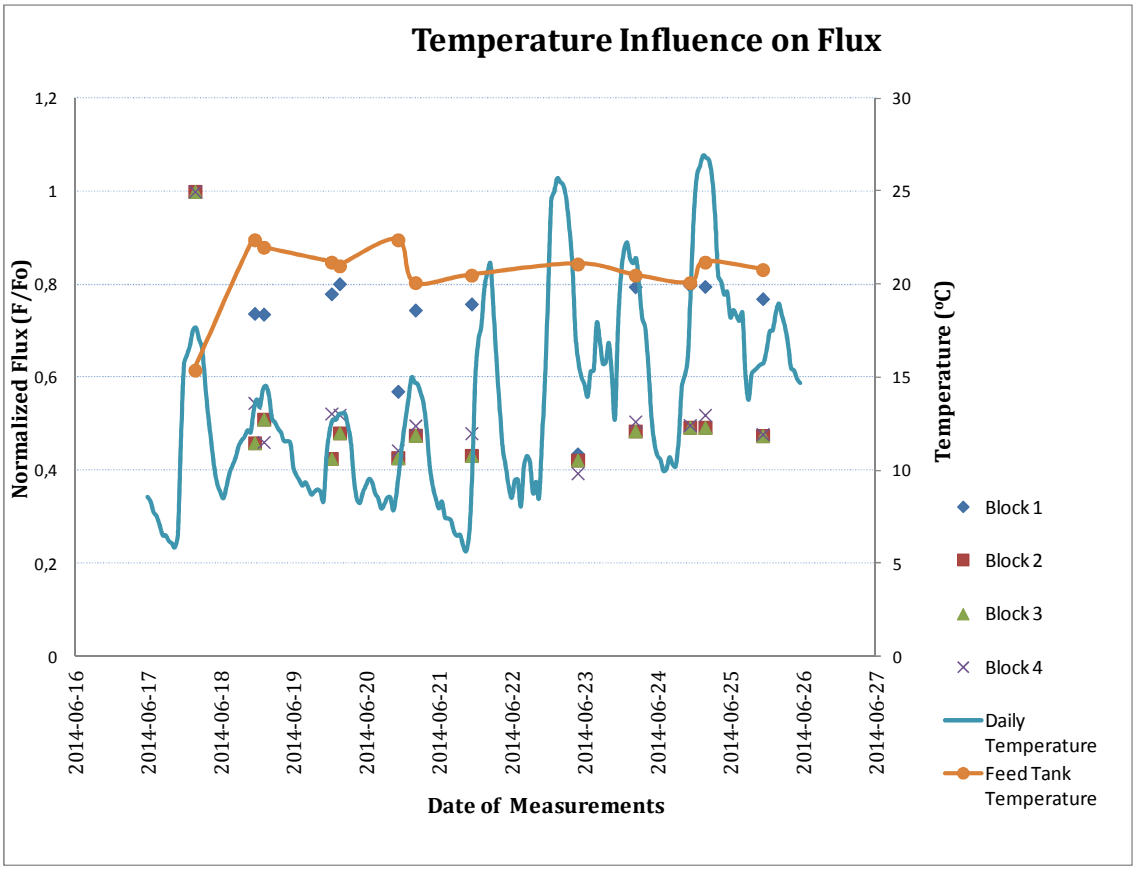


Figure 4-5: Influence of daily temperature variations on the measured normalised flux for DI water

The observed zigzag pattern in the flux data is only observed when the ambient temperature is much lower than the regulated feed-water temperature. Some cooling might have taken place from the feed tank to the blocks, causing the temperature on the membrane surface to be slightly lower than the feed-water temperature. A temperature difference of no more than 4 ^{o}C caused the pattern.

4.2.2 Hydrodynamic influence on fouling

The effect of different cross-flow velocities was investigated to determine the effect on the fouling behaviour. During these runs, DBNPA was dosed continuously at a high concentration (7 mg/l based on feed flow) to determine if a constant flux can be maintained with limited influence of biological fouling. The duration of the runs was 7 days.

(a) Normalised flux results

The biological fouling (if any) was limited by the relatively high concentration of DBNPA dosed. Flux decline therefore gave an indication of other potential foulants (particulate, inorganic or organic) present in the feed water. The normalised flux at different cross-flow rates is shown in Figure 4-6.

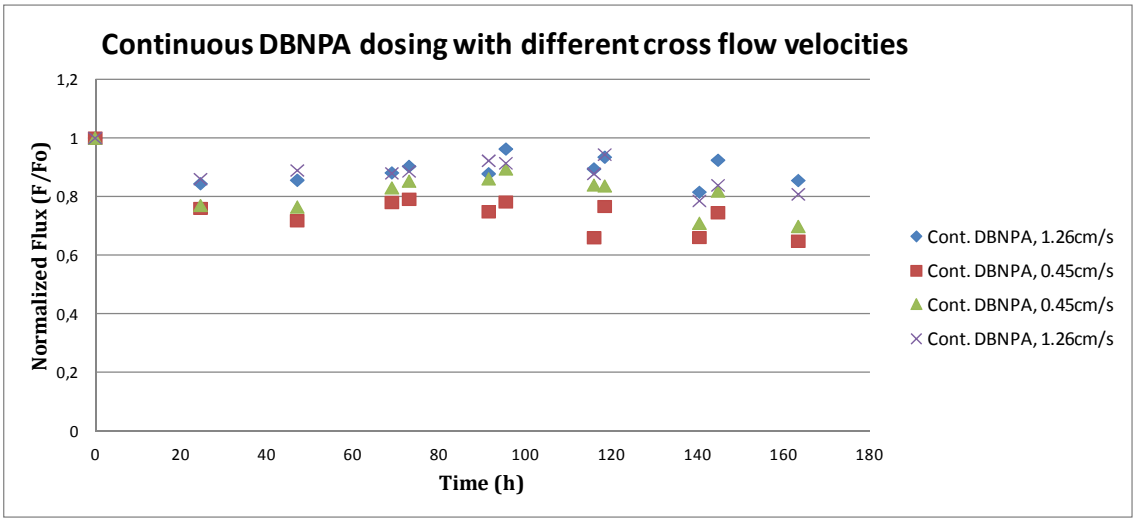


Figure 4-6: Normalised flux when continuous DBNPA is dosed with different cross-flow velocities. The low flow velocities are 0.45 cm/s and the high flow velocities are 1.26 cm/s. Two runs were carried out for each flow condition.

An initial flux decline was observed for both the higher cross-flow velocity (1.26 cm/s) and the lower cross-flow velocity (0.45 cm/s) for the first day, after which a more stable flux was obtained for two days before oscillations were observed. The order of the higher cross-flow velocity used in this study is the same as used in previous studies. However, it is still lower than cross-flow velocities used in industry (Li et al. 2012; Radu et al. 2012; Bucs et al. 2014). A flux recovery is then seen with both cross-flow velocities at around 100 h, followed by a secondary flux decline. The overall flux decline was also more for the blocks with the lower cross-flow velocity. The higher cross-flow velocity prevents the deposition of fouling in the feed water to a better extent than the lower cross-flow velocity. The nature of the initial fouling is also believed to be particulate/organic in origin. Particulate matter is still able to pass through the 1 µm-cartridge filter (Rudolfs & Balmat 1952). Ying et al. (2013) found two fouling stages when different shear rates were investigated. An initial organic fouling stage (confirmed by ATR-FTIR spectroscopy) was followed by a secondary biofouling stage. They found, however, that the flux decline for the higher cross-flow velocity was higher after around 150 h due to the nature of the biofouling formed or the higher organic loading rate. The dissolved organic carbon (DOC) was, however, much higher in their study than the measured total organic carbon (TOC) of the feed water in this study, which resulted in a faster flux decline in their study (Ying et al. 2013).

The flux recovery can also be attributed to the detachment of the fouling from the membrane surface due to possible channel formation as a result of the increased thickness of the fouling layer. This can be seen in Figure 4-7. This channel formation then caused higher localised flow velocities, thereby increasing the turbulence and removing the fouling layer. Fouling can still then occur on the clean spots later on. The zigzag temperature variations are again observed in the data.

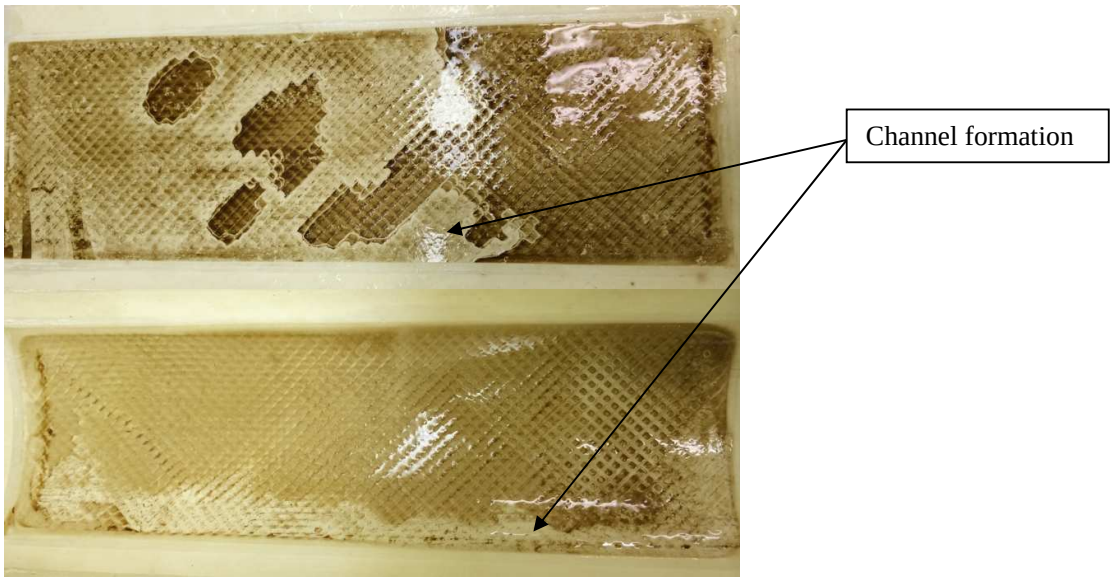


Figure 4-7: Visual observation of channel formations in the fouling layer on the two membrane coupons

The possibility of scale formation on the membrane was also investigated, causing the flux decline. A water analysis carried out by the CSIR, Stellenbosch (results in Appendix B) was used to calculate the LSI (Equation 2-16 and Equation 2-17) and thereby determine the potential risk of scaling. The LSI was calculated to be -2.82 when the recovery over the membrane was 20.2%. This indicates that the water has a tendency to dissolve scale from surfaces and cause corrosion as well. The LSI, however, provides no quantitative indication of the amount of scaling or dissolution that can take place. The CCPP could be used to provide a better indication of the kinetics involved.

(b) Biofilm parameters

The biofilm was removed from the membrane and homogenised after the fouling run was completed. The biofilm suspension was then analysed for protein and polysaccharide content, direct cell counts and CFUs. The results are shown in Figure 4-8.

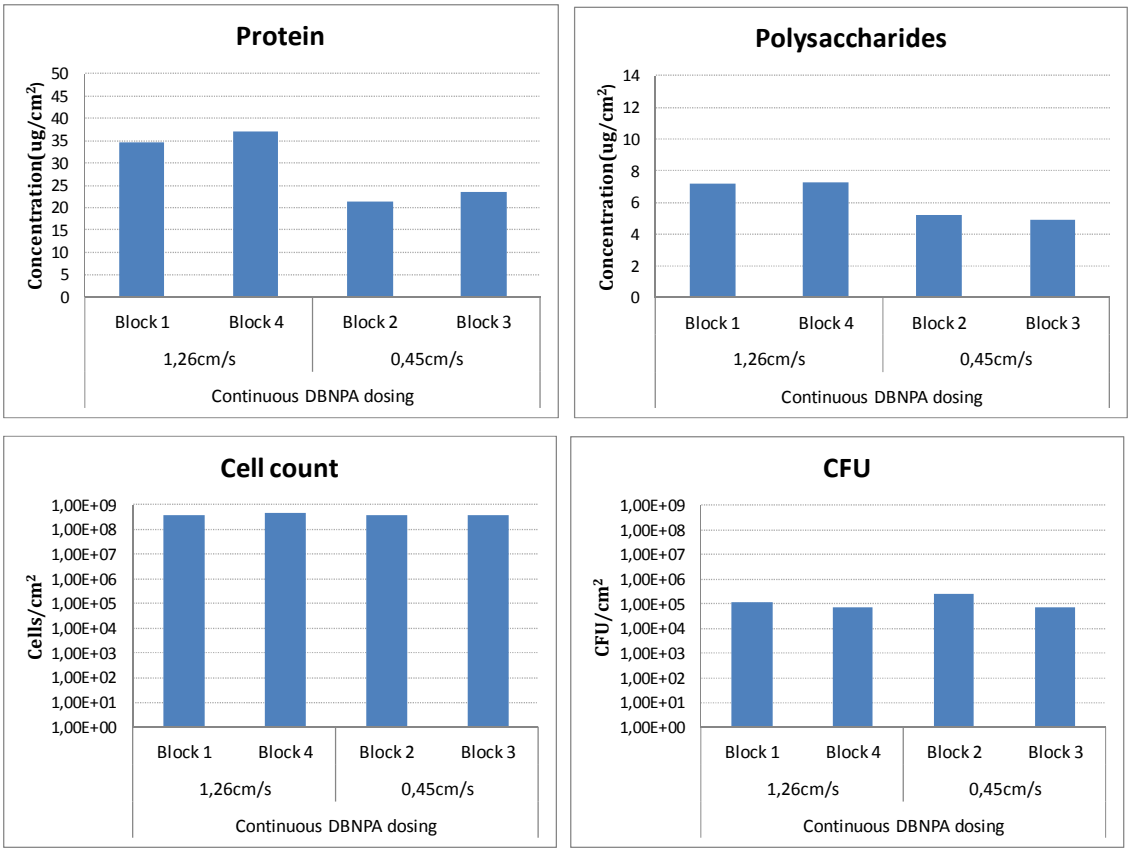


Figure 4-8: Analysis of biofilm formed under continuous DBNPA dosing and without supplementary nutrients at a low velocity of 0.45 cm/s and high velocity of 1.26 cm/s

The protein and polysaccharide content of the biofilm are higher for the 1.26 cm/s flow velocity compared to the lower flow velocity of 0.45 cm/s. A similar trend was observed by Dreszer et al. (2014). A higher cross-flow velocity will increase the shear rate on the biofilm as well as the nutrient loading rate. Therefore, the nutrient loading rate (even though no nutrients were added, the feed water still contained low concentrations of nutrients) for the higher cross-flow was higher, probably causing the higher protein and polysaccharide concentrations. However, the shear rate was also higher, which in turn reduced concentration polarisation. The net biofilm formation is therefore a balance between the biofilm detachment and biofilm growth (Dreszer et al. 2014).

Although there is a difference between the protein- and polysaccharide content of the biofilm developed under the different hydraulic conditions, it should be noted that the concentrations of these two parameters were very low compared to the work done by Dreszer et al. (2014). The protein- and polysaccharide concentrations measured by Dreszer et al. (2014) were between 50-100 µg/cm^2 and 50-100 µg/cm^2 respectively after only four days using unchlorinated tap water with supplementary nutrients as feed.

Suwarno et al. (2012), however, measured a protein concentration of around 40 µg/cm^2 and a polysaccharide concentration of around 40 µg/cm^2 when a model bacterium was used as feed organisms. When the results from this work are compared to work of Suwarmo et al. (2012) and Dreszer et al. (2014), it appears that only limited biofilm growth took place during the fouling runs and that the flux decline is influenced more by a different form of fouling.

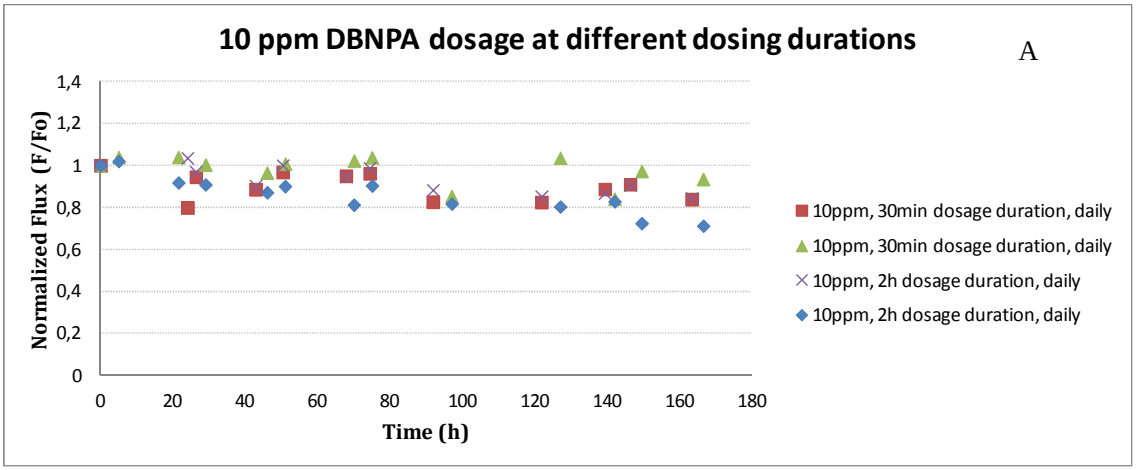
The cross-flow velocity did not visibly influence the CFUs and the total cell count for the different blocks. Although a notable flux decline was observed, low polysaccharide and protein concentrations (an indication of the amount of EPS) were measured. This result, as well as no noticeable difference between TCNs and CFUs, suggested that notable flux decline (Figure 4-7) could be attributed to another source of fouling that was non-biological in nature. It was then decided to carry out future runs only at higher cross-flow velocities to minimise the effect of particle deposition. It should also be noted that it was sometimes difficult to maintain an exact cross-flow velocity with only the pressure relief valve.

4.2.3 DBNPA dosing runs

Different dosing strategies were investigated by varying the dosage, dosing duration and frequency. Runs were carried out with no additional nutrient dosing and at a high cross-flow velocity. The duration of the runs was seven days.

(a) Normalised flux results

The normalised fluxes of the runs are presented in Figure 4-9.



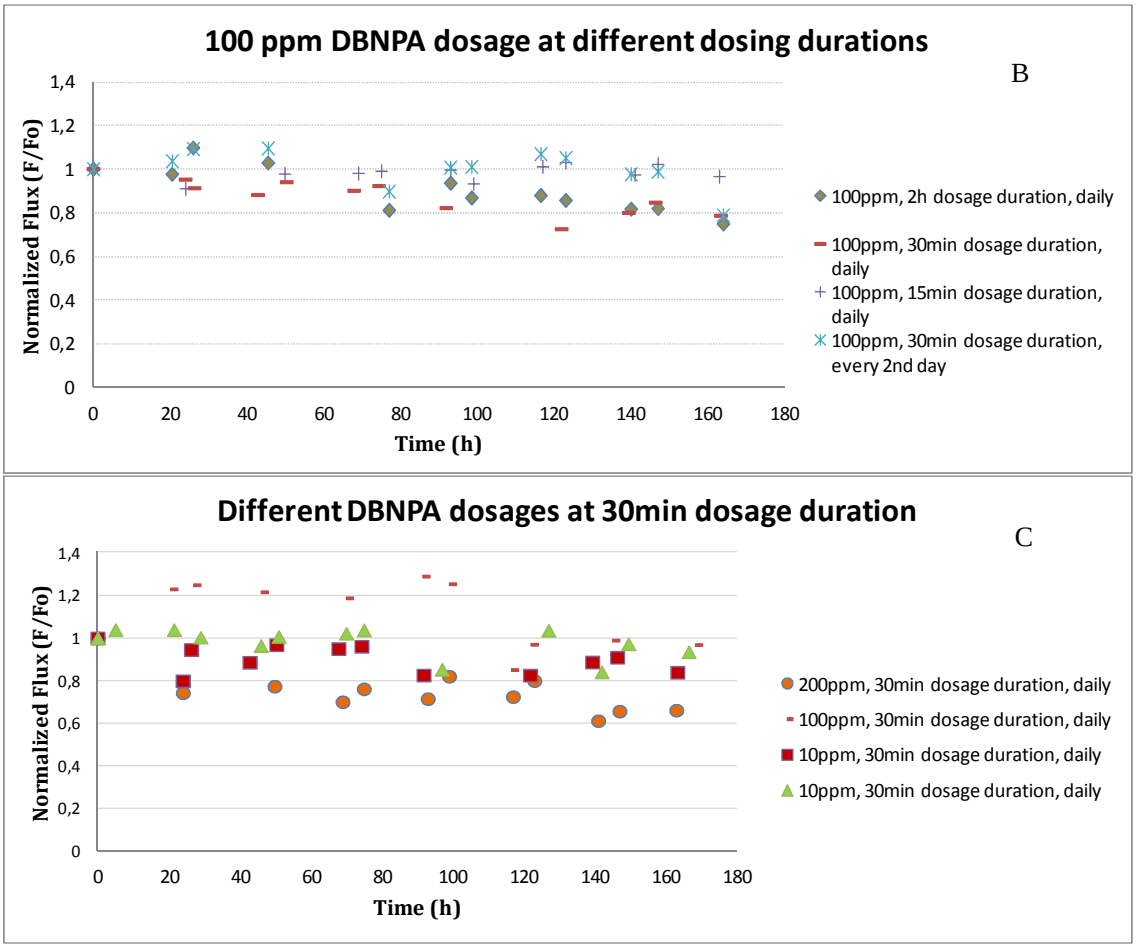


Figure 4-9: Normalised flux for different dosing strategies with no additional nutrient dosing

A downward trend is visible in the data, although a lot of scatter was observed. The scatter makes it difficult to distinguish between the different dosing strategies. There are a number of different causes that may have resulted in the data scatted. Possible reasons are:

- An unstable fouling layer (composed of biofouling and organic/particulate fouling) is formed. Detachment of the fouling layers occurs, which causes a wave-like function in the data. This is visible with a dosage of *10 ppm*, *30 min* duration, *daily dosing* frequency (Figure 4-9 (a)) and a dosage of *100 ppm*, *30 min* duration, *daily dosing* frequency (Figure 4-9 (b)). Flemming and Geesey (1990) also report in their study that primary, rapid attachment occurred, followed by an equilibrium phase, where cell growth and detachment occur.
- The unstable fouling layer is formed by mostly particulate/organic fouling. Biofouling is a more gel-like structure that would not be affected as easily as a fouling layer formed by the settlement particulate/organic material. The differences in normalised flux decline are therefore a measurement of the net fouling layer present, which is easily affected by channelling also shown in Figure 4-7.

- Zigzag variations in flux are possibly caused by extreme ambient temperatures.

(b) Biofilm parameters

The biofilm was removed from the membrane coupons and homogenised after the fouling runs were completed for the different DBNPA dosing strategies. The biofilm suspension was then analysed for proteins, polysaccharides, cell count and CFUs. The results are shown in Figure 4-10.

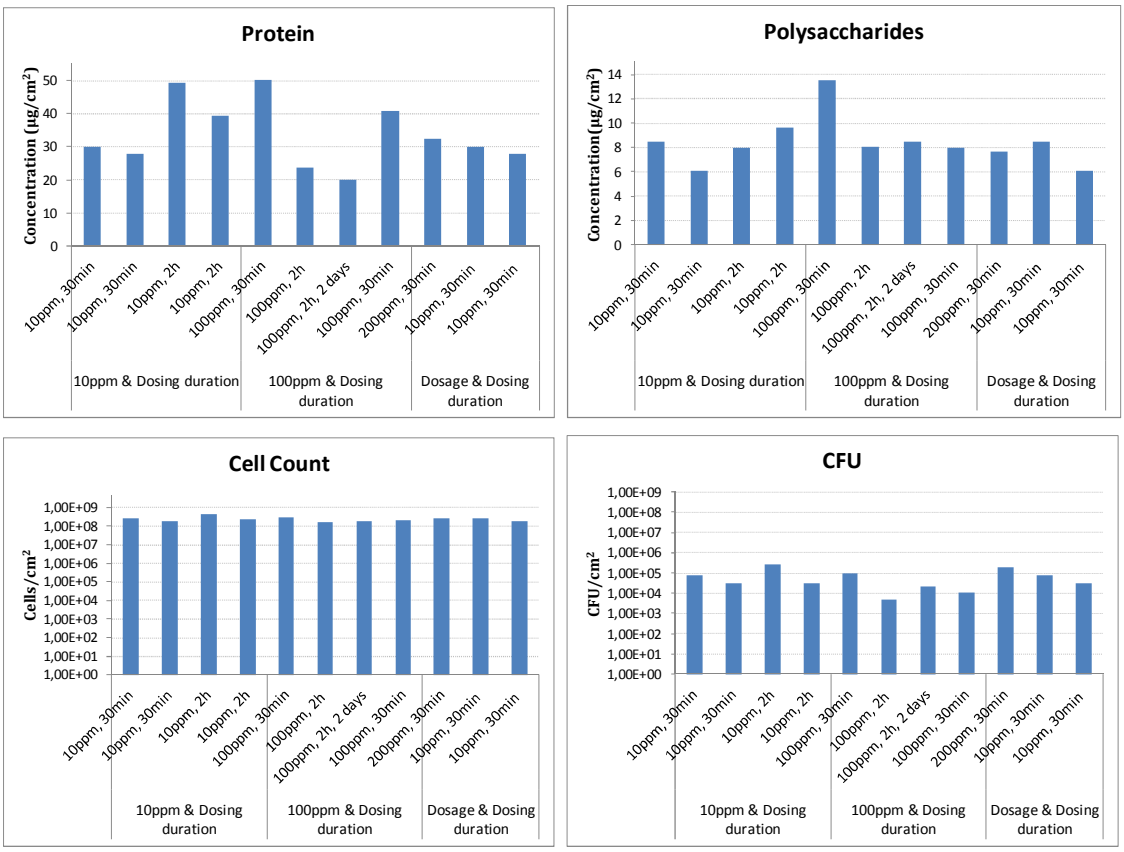


Figure 4-10: Analysis of biofilms formed on the membrane with different DBNPA dosing strategies with no additional nutrient addition

Again, no trends can be observed with respect to the different dosages and dosing durations with regard to the protein, polysaccharides, cell counts and CFU counts on the membrane surface. All the protein measurements varied between 19 µg/cm^2 and 51 µg/cm^2 and the polysaccharide contents between 6 µg/cm^2 and 14 µg/cm^2. The ranges are similar to values found by Suwarno et al. (2012) where a single bacterial strain (*Pseudomonas aeruginosa* PAO1) was used as model organism, which eliminates the effect of other fouling types. However, the CFUs measured an order of magnitude higher with the study carried out by Suwarno et al. (2012), compared to the results from this study. This could be attributed to the fact the DBNPA was dosed. The CFUs were also more than two orders lower than the cell counts, indicating that a substantial amount of cells were killed. The lack of trends is again an indication that the fouling on the membranes are not caused by biological growth and

secretion of EPS. When biological fouling is the dominant fouling, a larger influence of DBNPA (which is a fast-acting biocide used to control biofouling) will be observed, even if only slight trends.

The biofilm on the feed spaces was also analysed. The results are shown in Figure 4-11.

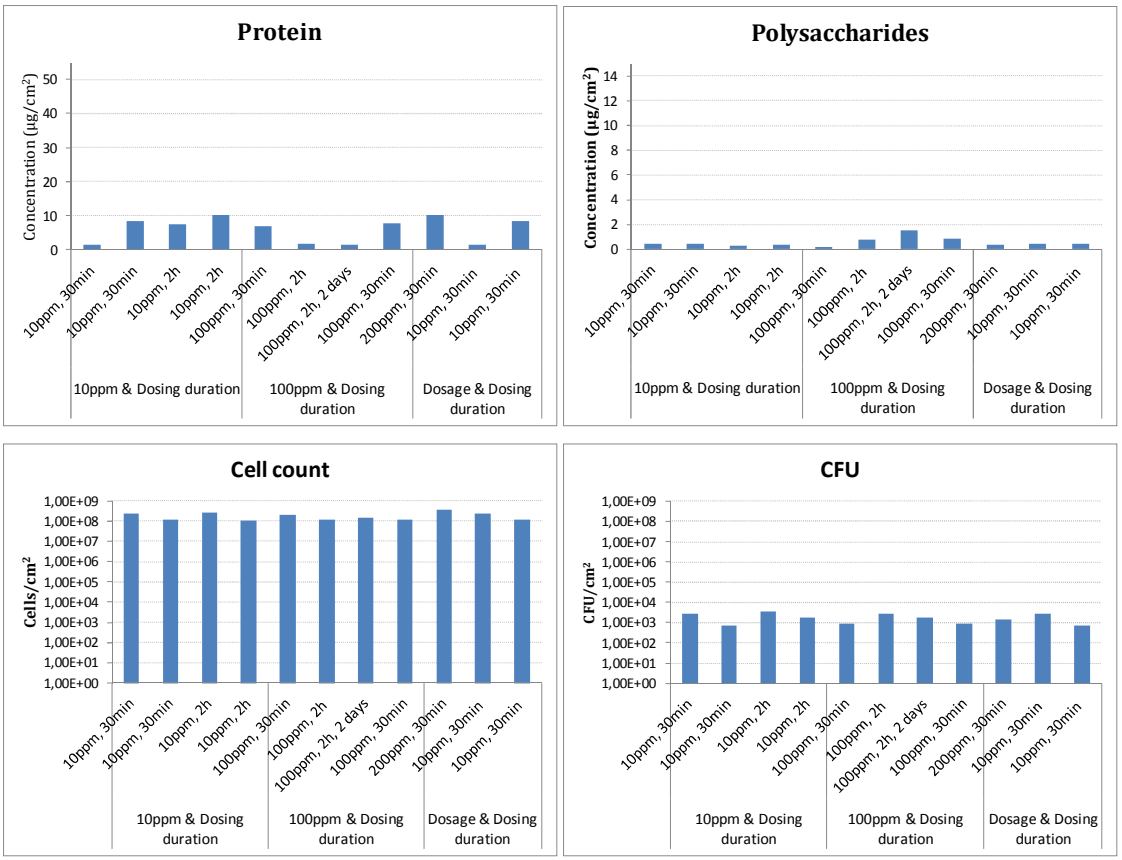


Figure 4-11: Analysis of biofilms formed on the feed spacers with different DBNPA dosing strategies with no additional nutrient addition

No trends were observed between the biofouling measured on the membranes and feed spacers compared to the different dosing strategy used. The biological parameters were also significantly lower on the feed spacer than on the membrane surface and very low in general. Furthermore, no observable fouling was visible on the spacers when removed from the blocks. Due to the nature of the feed spacer (90^{o} net design), deposition due to settling particle is also unlikely, therefore giving very low measured values on the feed spacers.

4.2.4 Biofouling runs without nutrient dosing – concluding remarks

It is clear from the measured parameters (flux, protein, polysaccharides, CFU and cell counts) used to quantify the fouling on the membranes and feed spaces that no clear trends were visible when compared to the DBNPA dosing. The amount of EPS formed and total cell count in this study were

also almost the same when compared to other studies, while the CFUs were lower. The dosing of DBNPA was responsible for the lower CFUs observed on the membranes.

Higher levels of biological fouling can be expected when supplementary nutrients are used to stimulate growth and no disinfection step is used in the preparation of the feed water. Microorganisms can also be in a stressed state after disinfection, causing a delay in growth and reproduction.

After the runs without nutrient dosing, an additional pressure-regulating valve was added before the blocks and pressured air was added to the pulsation damper to maintain the system pressure. The setup can be seen in Figure 3-2. This proved to give much better results. It was also decided to dose nutrients to aid biofilm formation.

4.3 Bio-fouling experiments with nutrient dosing

After completing the experiments without any nutrient addition, the influence of different dosing strategies was investigated when nutrients were added to the feed water. Slug dosing of the nutrients was performed daily to prepare a concentration of 100 µg/ℓ organic carbon in the feed-water tank. The composition of the added nutrients is discussed in Section 3.3.2. The runs were also carried out for nine days compared to the seven days used in the previous section to observe possible trends better. A summary of these runs is provided in Table 4-2.

Table 4-2: Description of fouling runs with nutrient dosing

Run	Feed water	Run Time (days)	Block	Dosing (dosage, duration, frequency)
7	Tap water + 100 µg/ℓ C	9	1	100 ppm, 30 min, daily
			2	100 ppm, 30 min, every 2nd day
			3	Control
			4	10 ppm, daily
8	Tap water + 100 µg/ℓ C	9	1	100 ppm, 2 h, daily
			2	100 ppm, 2 h, every 2nd day
			3	Control
			4	10 ppm, 2 h, daily
9	Tap water + 100 µg/ℓ C	9	1	10 ppm, 2 h, every 2nd day
			2	10 ppm, 30 min, every 2nd day
			3	Control
			4	10 ppm, 30 min, daily

The data are grouped together to compare the influence of dosing duration and dosage on the flux decline. The influence of dosage at a dosing duration of 30 min and 2 h is compared first, followed by

the influence of dosing duration with a DBNPA dosage of 10 ppm and 100 ppm. The measured biological parameters measured for these flux-decline data are then discussed.

4.3.1 Flux-decline data

(a) Flux decline compared at different dosing durations

The normalised flux-decline data are compared in this section at dosing durations of 30 min and 2 h at different DBNPA dosages.

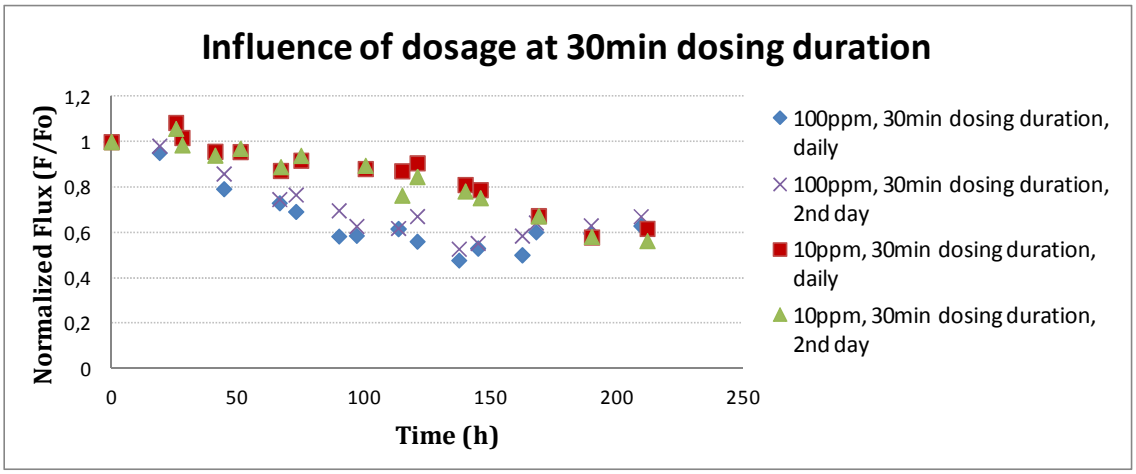


Figure 4-12: Flux decline observed when DBNPA is dosed at different concentrations for 30 min

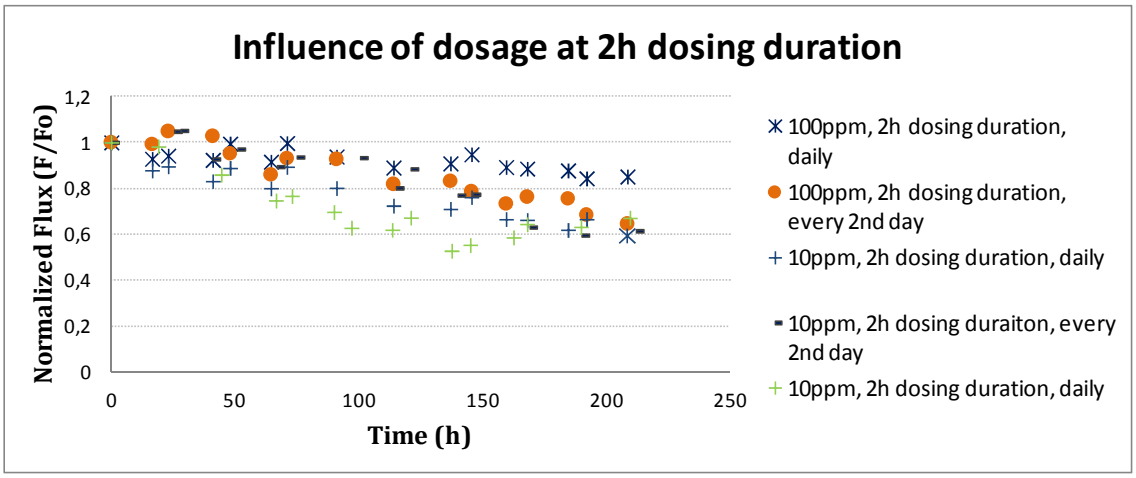


Figure 4-13: Flux decline observed when DBNPA is dosed at different concentrations for 2 h

The total normalised flux declined to around 60% of the initial flux at the end of the run period of nine days, except for the 100 ppm DBNPA dosage duration of two hours. A gradual flux decline was observed with this dosage (100 ppm) and duration (2 h) with a total normalised flux decline of only 15% after nine days.

Counter intuitive results are seen in Figure 4-12 during the 30 min dosing duration tests. It appears that the 10 ppm dosage for 30 min during both dosing frequencies (daily and every second day) performed much better to control biological fouling than the 100 ppm dosage for 30 min during both dosing frequencies (daily and every second day). It would be expected that the higher concentration of DBNPA would provide better biological control, resulting in a slower flux decline. Slight variations in the feed water can be responsible for the observed variations, since these two data sets were not recorded simultaneously. During every run, a control block in parallel was used to account for these variations.

The flux-decline data compared to the control blocks are shown in Figure 4-14.

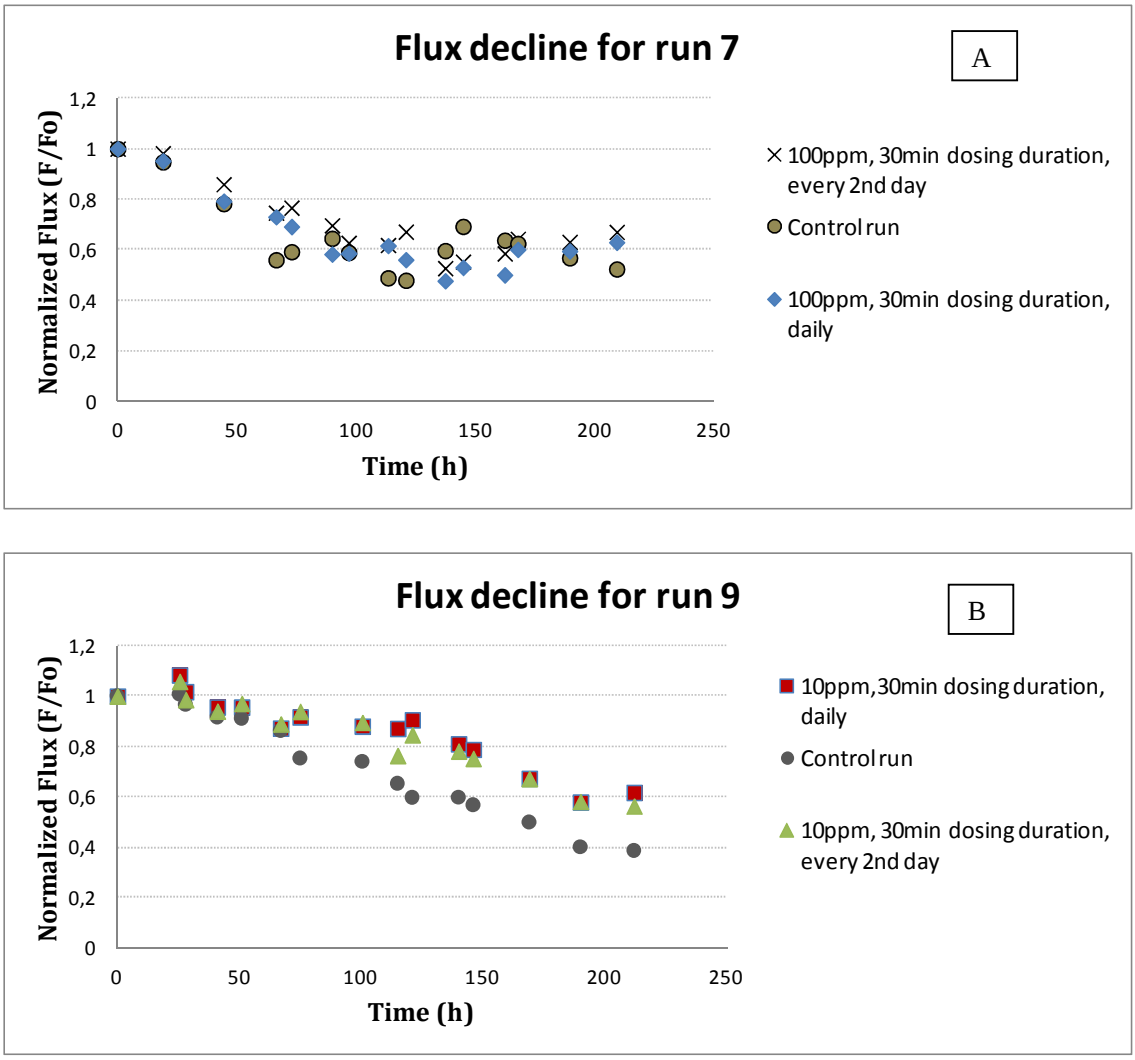


Figure 4-14: Dosing data compared to the control blocks

A slower normalised flux decline is observed in Figure 4-14 (B) with a 10 ppm DBNPA dosage, 30 min duration with both frequencies (daily and every second day). This is only a slight improvement, with no visible difference between the different dosing frequencies.

No apparent improvement is however observed in Figure 4-14 (A) when 100 ppm DBNPA is dosed for 30 min. A fast, normalised flux decline is observed for the control block, after which some flux recovery is observed. This trend continues until the end of the run to form a wave-like normalised flux decline. This wave can be characterised by a fast primary flux decline followed by a plateau that is reached. This plateau is associated with an equilibrium reached between attachment and detachment of microbial growth on the membrane surface. This was also observed by Characklis (1991). Channel formation may also have enhanced the biofilm detachment, improving the flux for a while after which fouling occurred again. The total possible normalised flux decline for the control block is therefore not available. The efficiency of the 100 ppm DBNPA dosage for a duration of 30 min for both frequencies is therefore difficult to determine, although it is expected that there would have been an improvement. The 100 ppm and 10 ppm DBNPA dosage for the 30 min during both frequencies can therefore not directly be compared for these two runs.

(b) Flux decline compared at different dosages

The normalised flux-decline data are compared in this section when 10 ppm and 100 ppm were dosed at the different dosing durations.

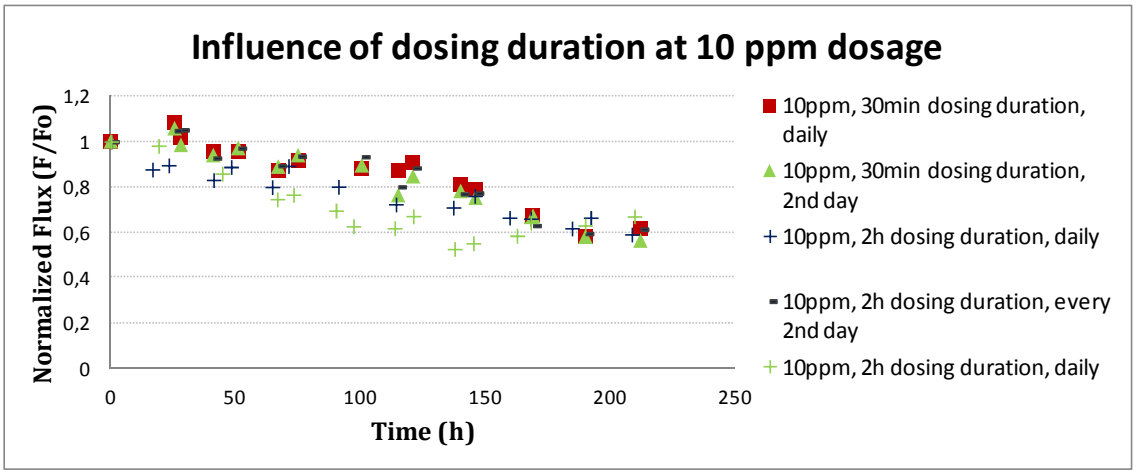


Figure 4-15: Flux decline observed when DBNPA is dosed at 10 ppm at different dosing durations

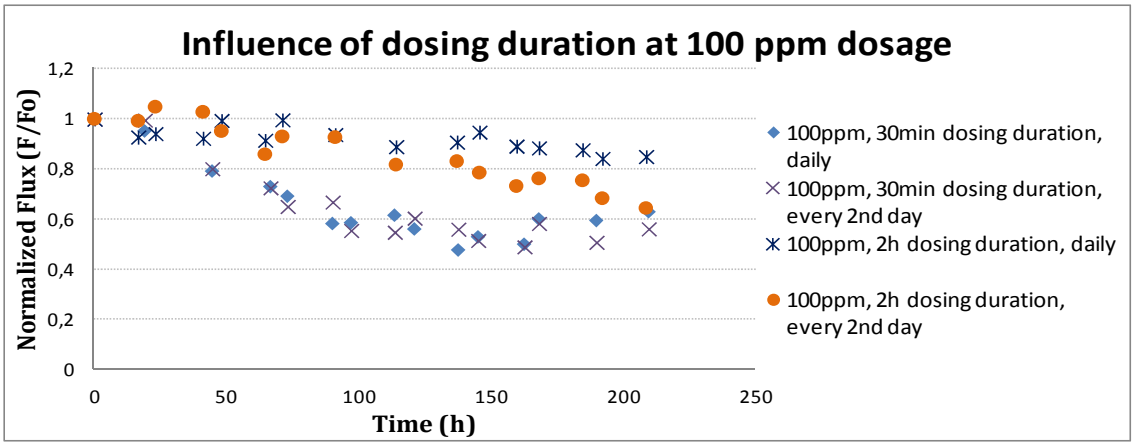


Figure 4-16: Flux decline observed when DBNPA is dosed at 100 ppm at different dosing durations

The same overall flux decline (to around 60%) is observed when a DBNPA dosage of 10 ppm is applied for different durations (Figure 4-15). A delayed flux decline is observed with some of the dosing durations, however the same overall flux decline was still observed after nine days.

When a dosage of 100 ppm is compared at different dosing durations, it is clear that dosing for 2 h every day is able to control the growth the best, as shown in Figure 4-16. This is also observable in Figure 4-13, although not that clear. Dosing 100 ppm DBNPA every second day was also able to control the growth to some extent, but after around 80 h, a faster flux decline was observed. The total flux decline was around 60%, which is almost the same as the runs where dosing was only done for 30 min It is clear that the longer dosing duration with a high enough concentration (even if dosing was done every second day), was more effective than the shorter dosing durations.

4.3.2 Biological parameters

The measured biological parameters (polysaccharides, protein, total cell count and CFU) are shown in Figure 4-17 to Figure 4-20 when DBNPA was dosed at 10 ppm and 100 ppm for 30 min and 2 h.

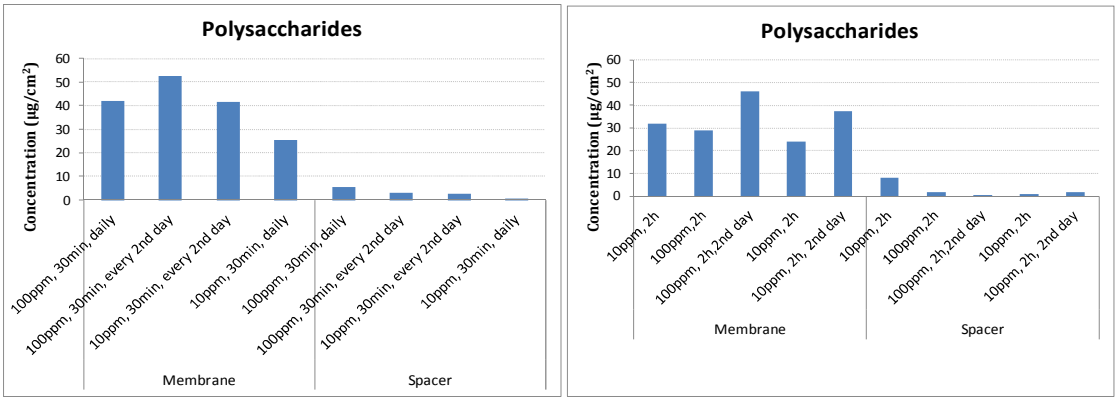


Figure 4-17: Measured polysaccharide concentrations on the membrane surface and feed spacer

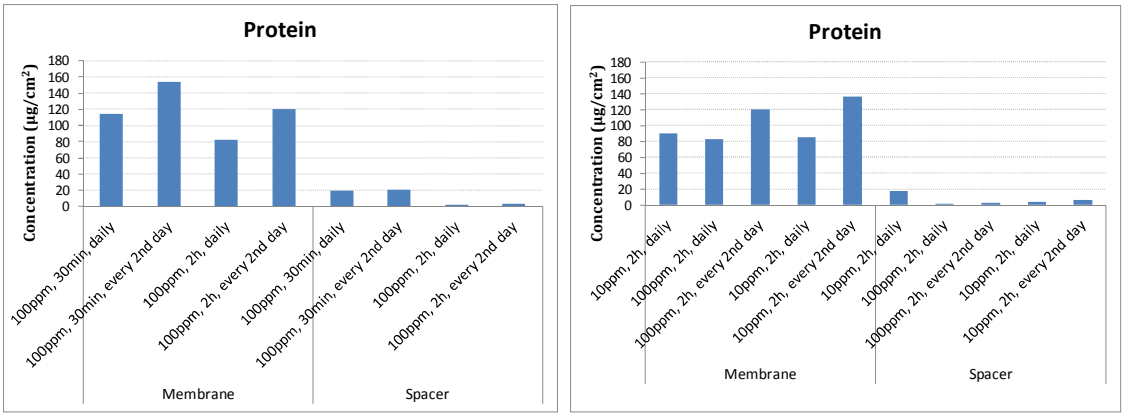


Figure 4-18: Measured protein concentrations on the membrane surface and feed spacer

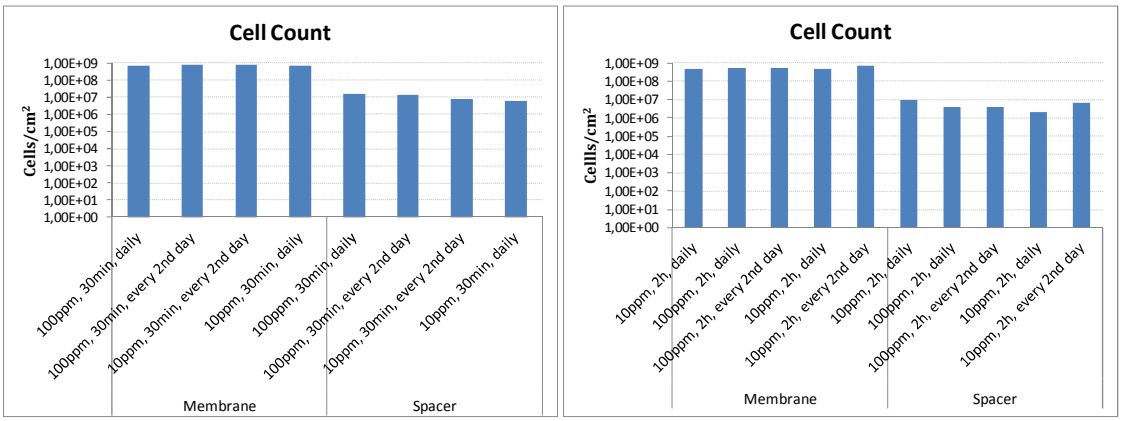


Figure 4-19: Total cell count on the membrane surface and feed spacer

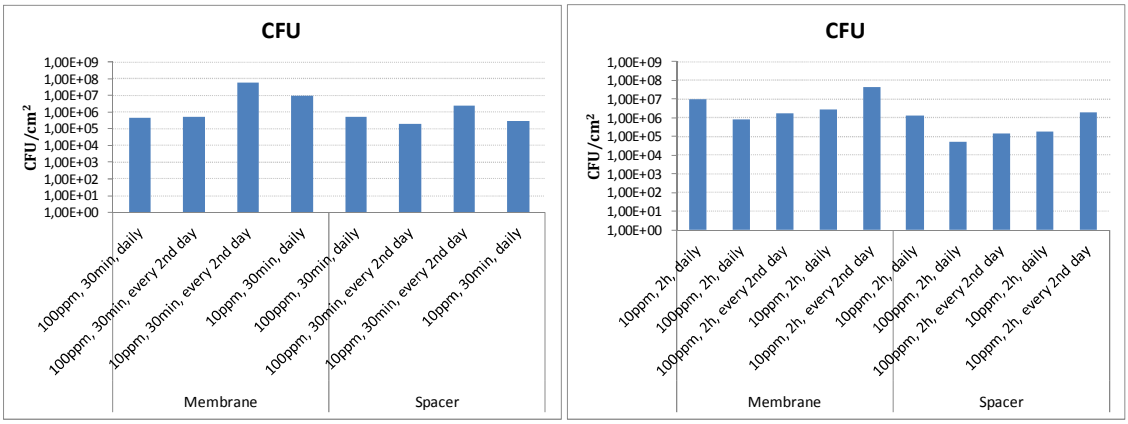


Figure 4-20: CFUs on the membrane surface and feed spacer

The polysaccharide concentration on the membrane varied between 24 µg/cm^2 and 53 µg/cm^2 and for the spacers between 0.65 µg/cm^2 and 5.54 µg/cm^2. The protein concentration on the membrane varied between 82 µg/cm^2 and 154 µg/cm^2 and for the spacers between 2.1 µg/cm^2 and 21.4 µg/cm^2. These values are much lower than the average values measured on the membrane for the control runs, which

were 68.1 µg/cm^2 for the polysaccharide concentration and 230 µg/cm^2 for the protein concentration. The much lower polysaccharide and protein concentrations measured when DBNPA was dosed are a very good indication that the microbial growth is suppressed, although no definite trends are observed between the dosing strategies.

The protein and polysaccharide concentration measured for dosing 100 ppm for 2 h every day is, however, still the lowest compared to the other dosing strategies. This, together with the flux-decline data, confirms that this was the best dosing strategy. It is later shown in this study that protein and polysaccharide concentration can be related to system performance (flux decline). This is also the reason why there are no clear differences between the dosing strategies, since the fluxes measured at the end of most of the runs were around 60%.

The cell count on the membrane surface for all the different dosing strategies was of the same order of magnitude and almost two orders of magnitude more that the cell count from the feed spacer. This, together with the protein and polysaccharide concentrations of the membrane and feed spacer indicates that most of the microbial growth took place on the membrane surface. Concentration polymerisation could have been one of the factors contributing to higher microbial growth on the membrane surface by increasing the nutrient concentration directly at the surface.

No distinct differences can be observed between the CFUs for the different dosing strategies. However, the number of CFUs is more than an order of magnitude lower than the cell count. This may indicate that the biofilm consisted of a large percentage of dead cells (killed by the DBNPA or by natural causes) that only a small percentage of the cells could be cultured in the laboratory, or that the CFUs were slightly underestimated (caused by the cell clumps).

There is also not a large difference between the CFUs on the feed spacer and on the membrane surface, although large differences are observed between the membrane and spacer when proteins, polysaccharides and cell counts are compared. A possible explanation is that the measured CFUs are present in the water surrounding the membrane and feed spacer, thus giving the same counts, while most of the cells in the biofilm are dead. However, a difference was observed between the CFUs in the feed spacer and membrane when no DBNPA was dosed (Figure 4-26), indicating that a large portion of live cells was still present in the biofilm.

4.3.3 Additional exploratory runs over extended periods

Additional runs were carried out to determine if a relatively stable flux could be maintained for a longer time with either a higher dosage (200 ppm), increased dosing frequency (twice a day), or an intermediate dosing duration (1 hour). The influence of the different parameters was all tested during the same experimental run. The results are shown in Figure 4-21. During the experiment, a number of

power cuts were experienced, which largely influenced the experiments. The power cuts were a result of load shedding and maintenance to a nearby substation, resulting in almost a weekend without continuous power supply. This also caused a shortage of compressed air, which made it very difficult to maintain the pressure. The influence of power failures is illustrated in Figure 4-21. The power failures occurred between 60 h and 150 h. It was then decided to extend the run period to 11 days to see if possible trends emerge from the data.

It is clear in Figure 4-21 that power outage had a large influence on the data. Although not confirmed, the increase in flux from 50 h onwards is probable due to the soaking of the fouling layer with no flux or cross flow to provide shear resistance. This may have caused the fouling to detach, resulting in a flux gain. This is followed by a sharp flux decline for the control block, with only slight differences visible between the different dosing strategies. A dosing frequency of twice a day also appears to be slightly more efficient than a single dosing per day for the same amount of time. This was only visible between 160 h and 200 h, after which the same flux decline was again observed. A stationary phase was also observed (from 150 h to 210 h) where a plateau was reached for the blocks where dosing was done. This is then followed by a steep flux decline. This flux decline was only observed with the runs, which lasted longer than nine days.

A few possible factors can contribute to the sudden flux decline. The threshold could have been reached for microbial growth on the membrane itself, causing uncontrollable growth (Flemming et al. 2011). The growth on the feed spacers could also have reached a point where severe channelling occurred, preventing a fresh supply of water from reaching certain parts of the membrane surface. The higher EPS content on the feed spacer is shown in Figure 4-22, which is indicative of this increase in fouling.

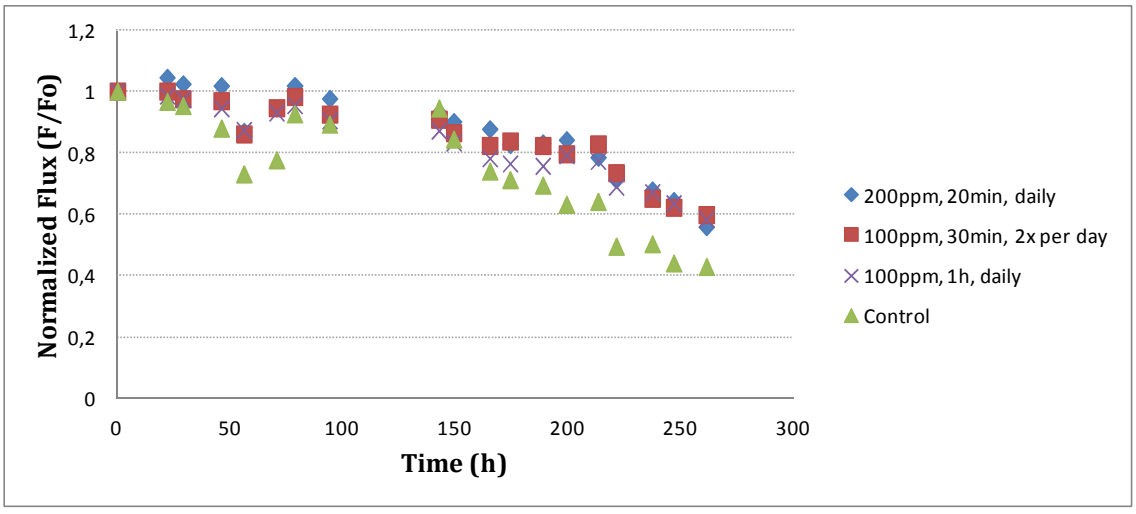


Figure 4-21: Influence of higher dosages, increased dosing frequency and intermediate dosing durations on flux decline

The measured biological parameters for the runs discussed above are shown in Figure 4-22. There is almost no difference visible between the protein concentrations for the different dosing strategies. The lowest concentrations were observed with dosing twice a day. The same was observed with the polysaccharide concentrations. The feed spaces showed significantly higher protein and polysaccharide concentrations compared to the other runs, which only lasted for nine days. As already mentioned, this indicated much more biological growth on the feed spacer, which may have been the cause of the fast flux decline.

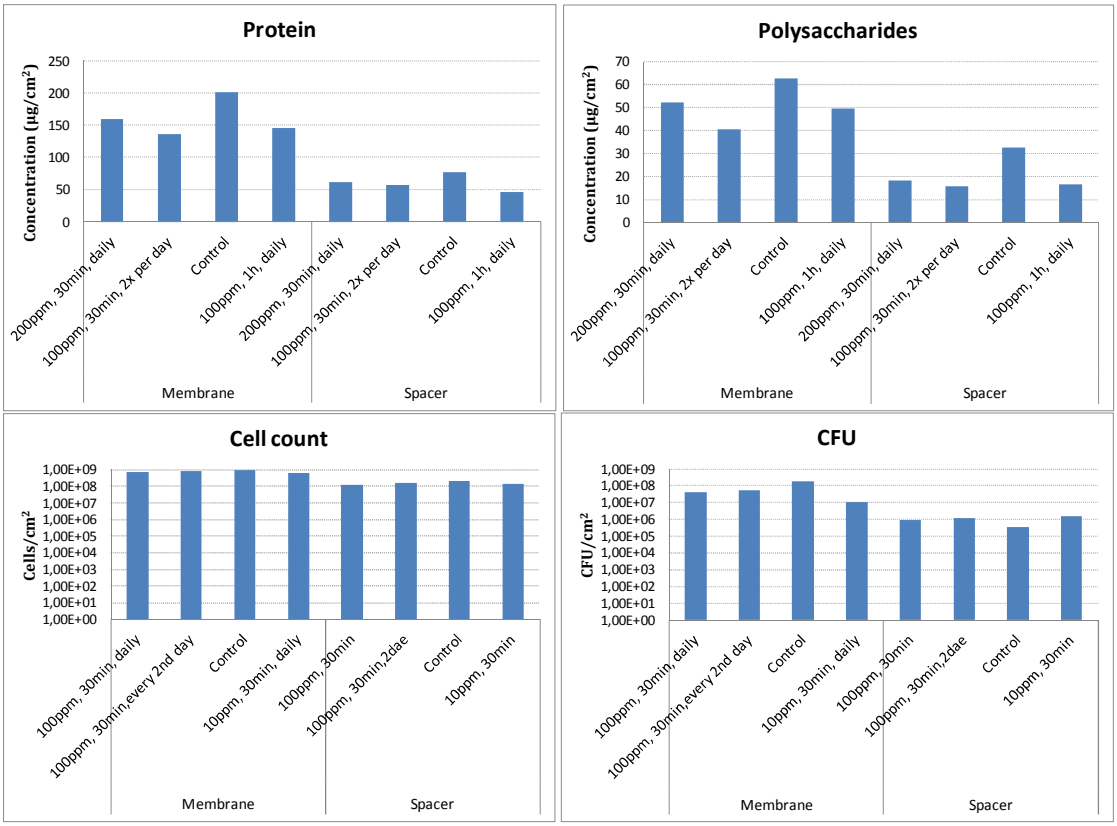


Figure 4-22: Influence of higher dosages, increased dosing frequency and intermediate dosing durations on biological parameters

The cell counts for the membrane and feed spacer are also of the same order (10^8), which is not the case with the other runs, which only lasted nine days. This indicated increased microbial activity on the feed spacer. A large difference is still observed with the CFU between the membrane and feed spacer. Some of the cells on the membrane surface might be better shielded by the EPS from the DBNPA dosing, causing the higher CFU counts.

4.3.4 Fouling runs with nutrient dosing – concluding remarks

The nutrients added to the feed water increased the microbial growth significantly. DBNPA dosing mostly reduced the amount of biofouling, with the 100 ppm dosage, 2 h-duration every day being the

most effective, followed by the same dosage and duration, every second day. It was however not able to prevent biofouling completely. No visible differences were observed with the 10 ppm dosage, regardless of the dosing duration. Although Grobe et al. (2002) have found that treating a biofilm with a higher concentration is more effective than an extended soaking time at a lower concentration this was not observed with this data.

The total flux decline of most of the runs was the same at the end of the run. This made it more difficult to differentiate between the biological parameters measured for the different dosing strategies used. After this steady plateau had been reached, a secondary decline was observed. It is recommended that the biological parameters should be measured before the plateau is reached. After the secondary flux decline, almost no differentiation is visible between the different dosing strategies.

4.4 Repeatability of control runs

During each run, one of the four parallel blocks was used for control purposes. The control block was operated under the same conditions as the other three blocks and received the same feed water, but no dosing strategy was tested. This was done to compare the different dosing strategies to a control block when slight variations in the feed conditions were suspected. The control block also made it possible to compare the different runs to each other.

4.4.1 Repeatability with no added nutrients

A number of runs were carried out without any nutrient addition. Tap water (filtered through GAC and 1µm cartridge filter) was used as feed water. Only the pressure relieve valve was used to regulate the pressure. This caused scatter in the data, which is shown in Figure 4-23.

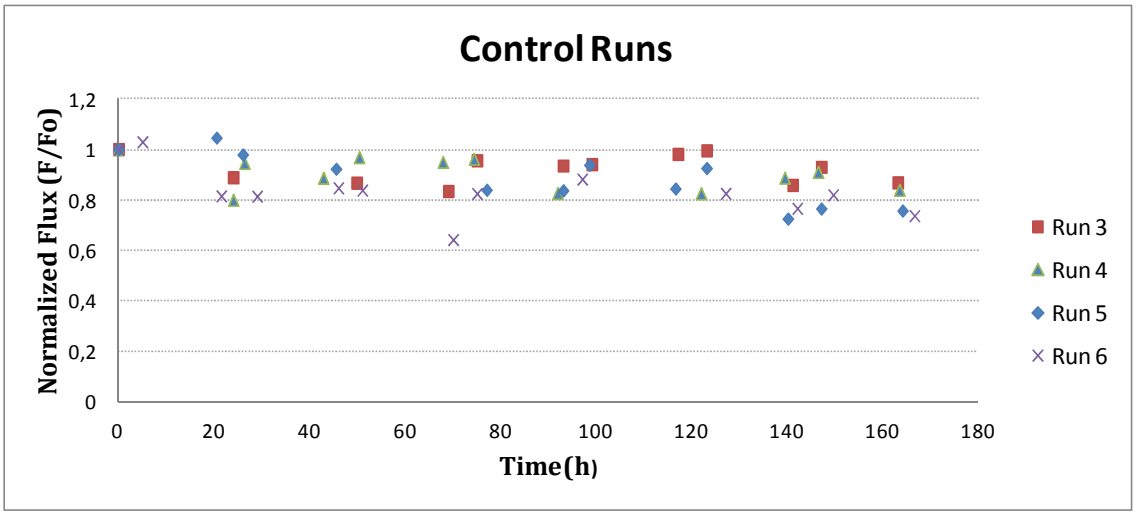


Figure 4-23: Flux decline observed for control blocks during each run with no nutrients added

Most of the data points are situated between 100% and 80% of the initial fluxed observed with an end flux decline of around 80%. A possible explanation is also that the fouling layer was a result of the deposition of particles. Changes in flow patterns then caused the detachment of particles, resulting in a flux gain. Although the feed-water temperature was regulated, ambient temperatures may have also caused slight variations in the data.

The biological parameters for the control runs were also analysed. This is shown in Figure 4-24. The average polysaccharide concentration on the membranes and feed spacers were 12.36 µg/cm^2 and 0.79 µg/cm^2 with a standard deviation of 2.85 µg/cm^2 and 0.25 µg/cm^2, respectively. The average protein concentration for the membrane and feed spacer was 34.68±7.13 µg/cm^2 and 6.37±4.73 µg/cm^2, respectively. The average cell counts for the membrane and spacers were $3.05x10^8$±$5.02x10^7$ cells/cm^2 and $1.34x10^7$±$6.42x10^6$ cells/cm^2, respectively and an average CFU $3.81x10^6$±$3.09x10^6$ CFU/cm^2 and $5.54x10^4$±$4.46x10^4$ CFU/cm^2 for the membrane and spacer, respectively. The CFUs on the spacer for run 6 were too numerous to count, making the exact amount unavailable. It was however more the 10^4 CFU/cm^2. The variations in biological parameters are caused by the same reasons mentioned above.

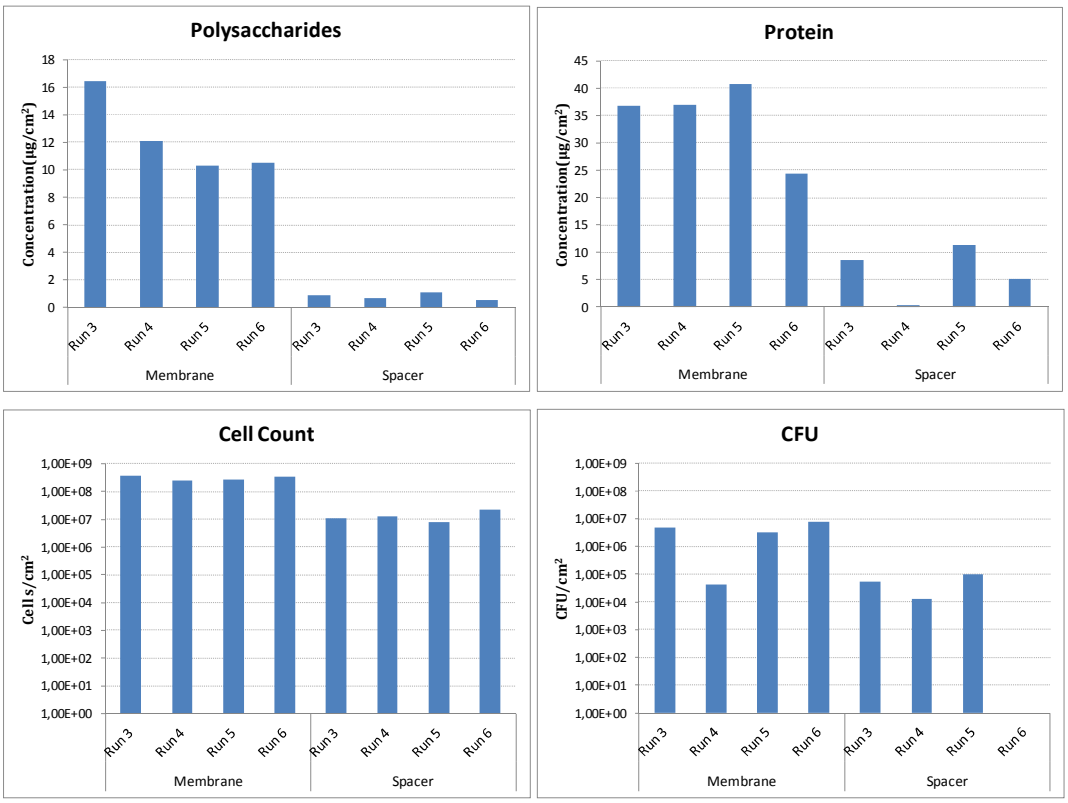


Figure 4-24: Biological parameters for the control runs when no nutrients were dosed

4.4.2 Repeatability with added nutrients

A number of runs were carried out with supplementary nutrient, with a control block in each run. Tap water (which was filtered through GAC and 1 µm cartridge filter) was used as feed water, with 100 µg/ℓ C added as nutrient source on a daily basis. More details on the nutrients can be found in Section 3.3.2. The normalised flux decline observed is shown in Figure 4-25. Large variations were observed in the data. During run 7, a wave-like pattern was observed in the data. This was probably caused by the formation of unstable biofilm. Detachment of the biofilm caused a flux increase, which was then followed by additional growth causing the decline. This process repeated itself until the end of the run. Flow channels within the block may have caused the unstable biofilm. This argument is supported by the measured protein and polysaccharide concentrations shown in Figure 4-26. The measured protein and polysaccharide concentrations are slightly lower on the membrane and much more on the feed-spacer side. Some of the detached biofilm from the membrane could have stuck to the feed spacer, resulting in the higher measured values.

The cartridge filter and some of the activated carbon were replaced prior to run 9. This could have contributed to the slower flux decline. Due to time constraints, only a limited number of runs with nutrients could be carried out. Additional runs may clarify the shape of the flux decline, since single factors contributed to the different shapes observed.

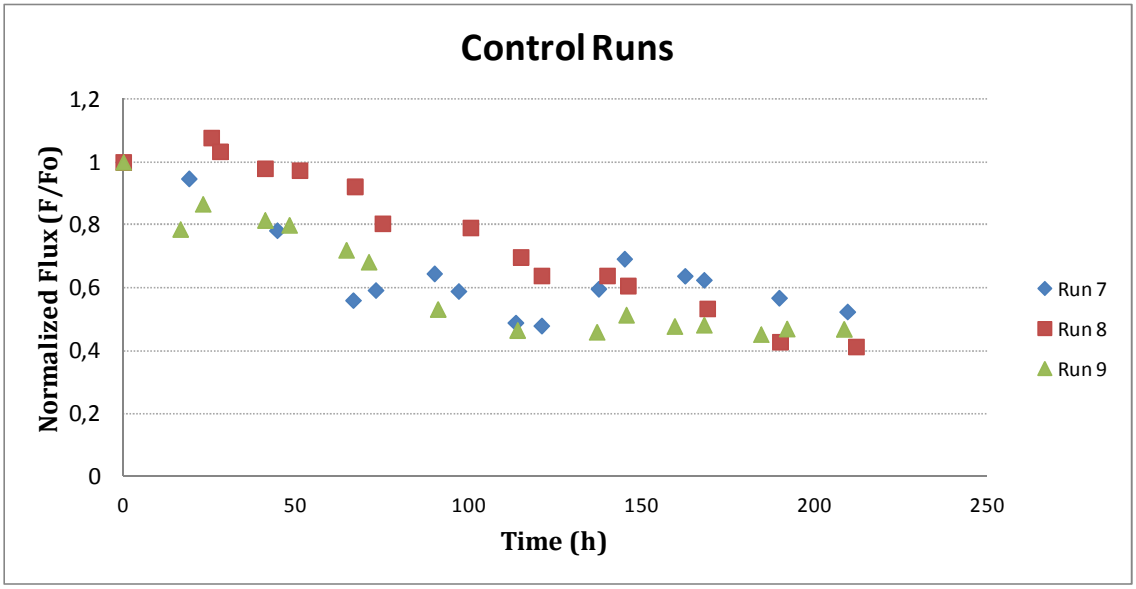


Figure 4-25: Flux decline observed for control blocks during each run with nutrients added, but no DBNPA

The biological parameters for the control runs were also analysed. This is shown in Figure 4-26. The average polysaccharide concentration on the membranes and feed spacers are 68.12 µg/cm^2 and 10.74 µg/cm^2 with a standard deviation of 1.73 µg/cm^2 and 14.05 µg/cm^2, respectively. The average

protein concentrations for the membrane and feed spacer were 230±36.94 µg/cm^2 and 32.73±35.52 µg/cm^2, respectively. The average cell counts for the membrane and spacers were 7.16x10^8±1.67x10^8 cells/cm^2 and 1.56x10^8±1.32 x10^8 cells/cm^2, respectively and the average CFU 9.72 x10^7±7.56x10^7 CFU/cm^2 and 1.63x10^5±1.72 x10^5 CFU/cm^2 for the membrane and spacer, respectively.

Although variations between the paths of the flux declines are observed, limited variations are observed within the final measured biological parameters. The protein and polysaccharide concentration for the feed spacers for run 8 and 9 are also almost the same. There is also very little variation between the cell counts and CFU for the different runs on the membrane and feed spacer, with only the cell counts for the feed spacer on run 7, which is lower than the rest.

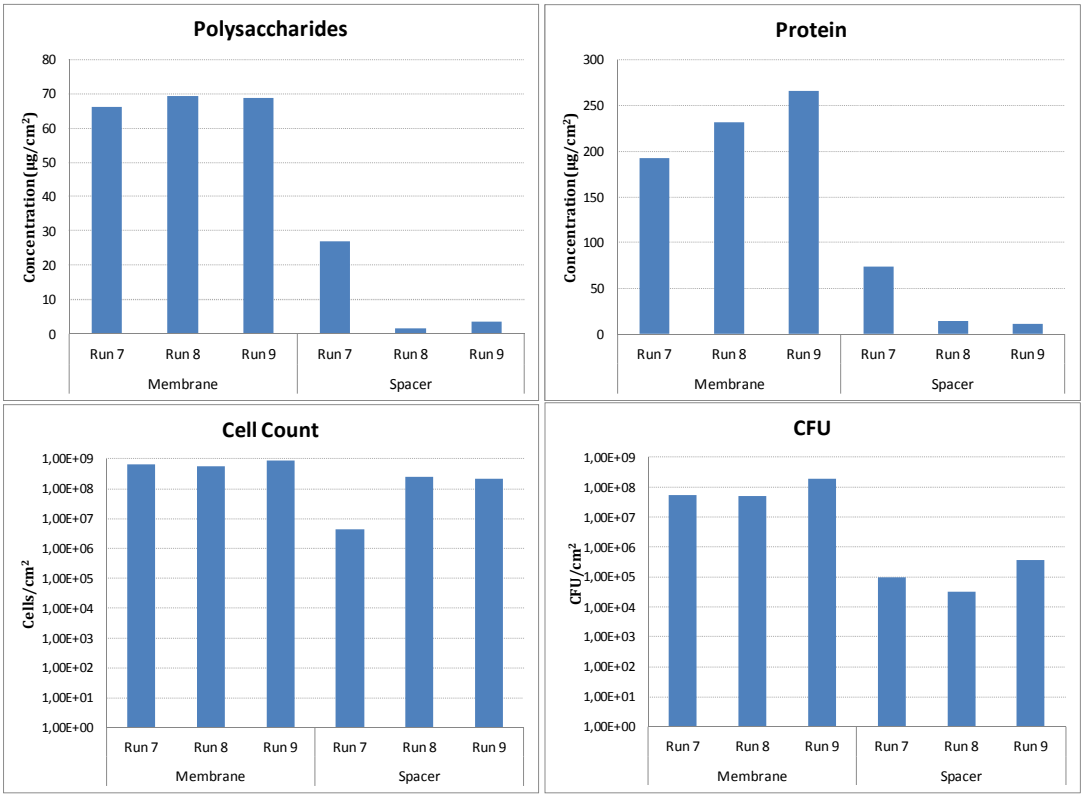


Figure 4-26: Biological parameters for the control runs when nutrients were dosed

4.5 Quantitative biofouling analysis

Quantification of biofouling is difficult, because no univocal quantification method exists to link operating conditions to biofouling (Vrouwenvelder et al. 2008). Pressure drop is usually used to measure the amount of fouling on the membrane; however, it is not exclusively linked only to biofouling. The pressure drop may also not be sensitive enough for early detection of biofouling. A relationship between a biological parameter and operating conditions is therefore necessary to be able to quantify the fouling as biological fouling. A number of biological parameters can be used for this task.

The results from the previous section are used to find a possible relationship between the biological parameters and process performance. Protein, polysaccharide concentration, cell count and CFU are used as biological parameters. The percentage flux decline is used as process parameter. This can be linked to pressure increase, which is the preferred measured variable on a plant. The percentage flux decline and biological parameters were measured at the end of the different DBNPA dosing runs. Only the biological parameters from the biofilm on the membrane surface were used and not the parameters on the feed spacer, because the flux decline is not directly linked to the amount of biological activity on the feed spacer. A better performance parameter to compare to the fouling on the feed spacer would be the pressure drop over the feed channel.

4.5.1 Effect of feed-water nutrients

100 µg/ℓ C acetate was added to the feed water daily for the additional nutrient dosing. The difference in biofilm formation was compared with and without nutrient addition. The measured flux decline was significantly higher ($p=2.27x10^{-7}$) for the runs with nutrient addition compared to the runs without nutrient addition. The biomass parameters were also significantly higher when nutrients were added. The polysaccharide contents increased almost four times, the protein content around 2.3 times, the cell count 1.5 times and the CFU 25 times. This increase was only observed when the runs with and without nutrients were compared to runs where the run time was seven days as well. The additional two days will cause an even higher increase in biological parameters measured.

It is clear that an increase in biodegradable components in the feed water will cause higher amounts of biofouling accumulation on the membrane surfaces. By monitoring the biodegradable components in the feed water, a possible early warning system for biofouling can be created before significant performance issues are observed.

4.5.2 Biomass concentrations on membranes

The DBNPA dosing used on the membrane prior to biofilm analysis is summarised in Table 4-1 and Table 4-2. The relationships between the normalised flux decline and protein, polysaccharides, cell counts and CFU are shown in Figure 4-27.

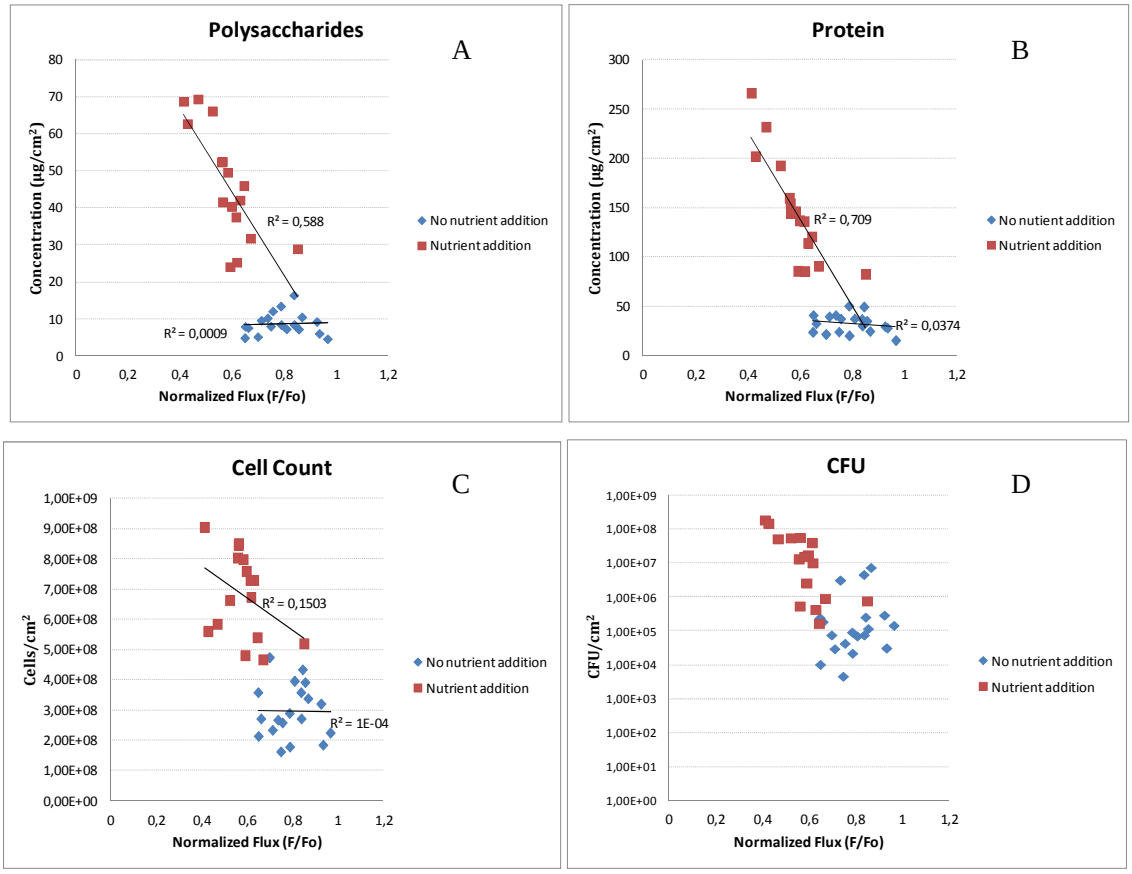


Figure 4-27: Relationship between normalised flux decline and protein (A), polysaccharide (B), cell counts (C) and CFU (D) measurements with and without substrate dosing

It is clear that there is a distinct difference between the data obtained from the fouling runs where nutrients were added and runs without added nutrients. Nutrient addition stimulated biofilm formation, causing an increase in the values measured for the biological parameters. The additional two days of operations are also responsible for the higher measured values. The ranges of measured biological indicators are also much larger when nutrients are dosed, compared to the ranges where no nutrients are dosed. The ranges of the different parameters measured are summarised in Table 4-3.

Table 4-3: Ranges of measured biological parameters with and without nutrient dosing

	No nutrients		Nutrient addition	
	Min	**Max**	**Min**	**Max**
Protein (µg/cm^2)	15.20	50.07	82.38	266.11
Polysaccharides (µg/cm^2)	4.61	16.44	24.11	69.40
Cell count (Cells/cm^2)	$1.62x10^8$	$4.73x10^8$	$4.67x10^8$	$9.04x10^8$
CFU (CFU/cm^2)	$4.67x10^3$	$7.48x10^6$	$1.65x10^5$	$1.84x10^8$
Flux Decline	0.65	0.97	0.41	0.85

With Nutrient Dosing:

The concentration of polysaccharides and proteins are more sensitive and accurate indicators than the normalised flux-decline measurements and have less scatter than the cell counts and CFU. Contrary to Vrouwenvelder et al. (2008), a reasonably significant relationship was found between the polysaccharide concentration ($p=0.0053$, $R^2=0.58$) and the protein concentration ($p=4.29x10^{-5}$, $R^2=0.71$) and the normalised flux decline respectively. When lower protein (concentration<100 µg/cm^2) and polysaccharide (concentration<30 µg/cm^2) are removed from the regression, a better fit will be obtained. The relationship will be very significant for protein concentration ($p=3.35x10^{-6}$, $R^2=0.89$) as well as polysaccharide concentration ($p=4.95x10^{-5}$, $R^2=0.79$). The lower protein and polysaccharide values are removed, since a range of flux-decline values did not correspond. Similar data analysis was done by Vrouwenvelder et al. (2008) to obtain a better fit.

A weak, but still significant relationship ($p=0.0011$, $R^2=0.54$) was also found between the CFU and normalised flux decline. This weak relationship can be attributed to a number of factors. Only a certain amount of cells is cultivable on agar, which gives an underestimation of the true amount of cells. There are also cell clusters visible, which also lead to an under-prediction of results. The CFU is further an indication of active biomass, which in turn can cause the secretion of EPS. Different DBNPA dosing strategies were investigated prior to biofilm analysis. The amount of active cells that were responsible for the biofouling could therefore not be quantified by heterotrophic plate counts.

However, the relationship found for the heterotrophic plate counts was better than the relationship found by Vrouwenvelder et al. (2008). No significant relationship ($p=0.14$) was found between the normalised flux decline and the total cell count. It is believed that the cell clusters observed during total cell count did not influence this relationship drastically. A large number of blocks were counted to mitigate the problem. The biofilm removal process was also the same throughout, making it more likely to cause an under-prediction, rather than influencing the entire relationship. A possible explanation is that all the cells (dead and alive) are included in the total count. The amount of cells responsible for the secretion of EPS is also unknown because the cells' active time is unknown. Kolari et al. (2003) have found that a low concentration of DBNPA stimulates the formation of a biofilm in bacteria that are commonly found in a paper machine. The DBNPA could also have had the same

effect on the biofilm. The secretion of EPS is therefore a function of DBNPA concentration and not necessarily of bacterial cell count. The types of bacteria found on the biofilms were not investigated. However, a significant relationship was measured by Vrouwenvelder et al. (2008) between pressure increase and total cell count. This might have been possible due to the fact that no cleaning was applied to the membranes in the study at least ten days prior to membrane removal.

Without Nutrient Dosing:

No significant relationships were found between the normalised flux decline and any of the biofouling parameters used. The significance for the protein, polysaccharide, total cell count and CFU are p=0.90, p=0.43, p=0.97 and p=0.47, respectively. A number of reasons can furthermore be attributed to this lack of a significant relationship. Biological fouling is not the only factor responsible for the fouling layer on the membrane surface. Organic/particulate fouling could have been present on the membrane, causing the flux decline. Another possible explanation is that a certain amount of biofouling must form before the fouling layer can be classified as biological fouling. Protein and polysaccharide values larger than 100 µg/cm^2 and 30 µg/cm^2, respectively seem to give relatively good results. Vrouwenvelder et al. also used ATP values higher than 1000 pg ATP/cm^2 for the correlations generated.

4.5.3 Interaction between biological parameters

The relationship between the different biological parameters used for biological fouling diagnosis is investigated in this section. A strong relationship between two parameters can possibly reduce the number of measured biological parameters, since an unknown value can be determined using inference. The relationship between the cell count and CFU is shown in Figure 4-28.

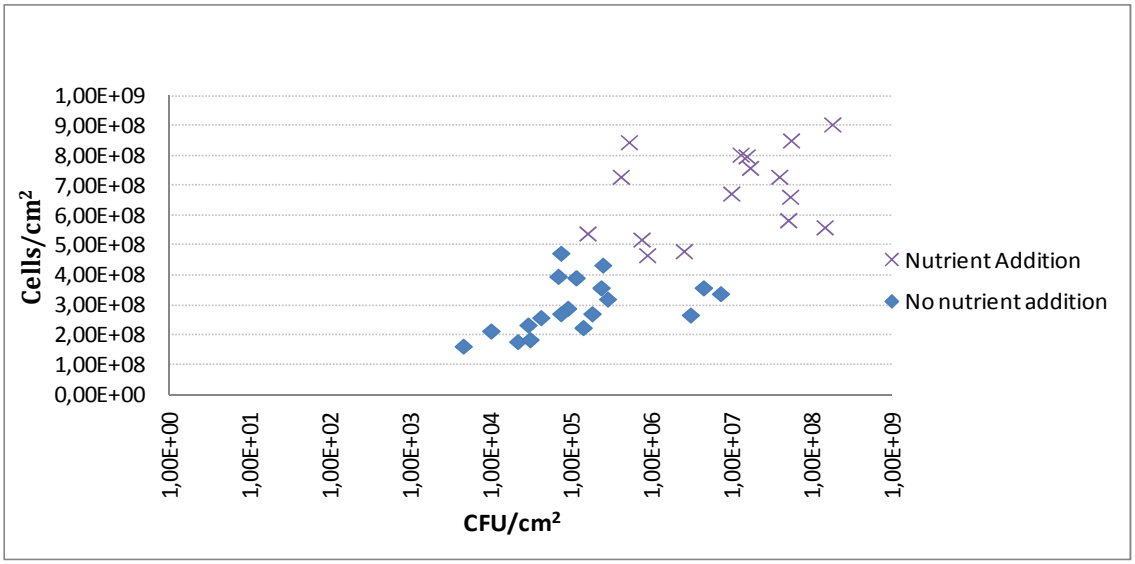


Figure 4-28: Relationship between cell counts and CFU with and without the addition of nutrients

A weak but significant (p=0.0025, R^2=0.25) relationship exists between the number of CFU and the cell count when the entire range of data is taken into account. The weak relationship can again be attributed to the observed cell clusters and the fact that only a certain percentage (shown in Figure 4-29) of the bacteria cell is culturable on agar. Using either one of these variables to predict the other one will yield poor results.

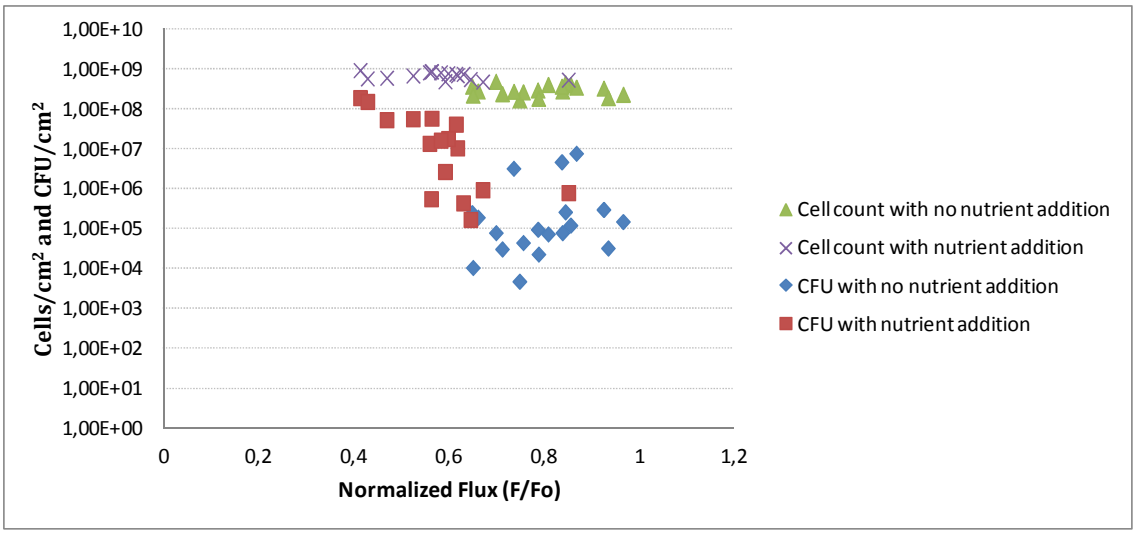


Figure 4-29: Difference in numbers between CFU and cell counts

The cell counts are almost an order of magnitude larger than the largest number of CFUs measured and almost five orders of magnitude larger than the minimum number of CFUs measured. This again confirms that a large portion of the cells in the biofilm is not measured by the CFU method.

The relationship between the protein and polysaccharide concentration is shown in Figure 4-30.

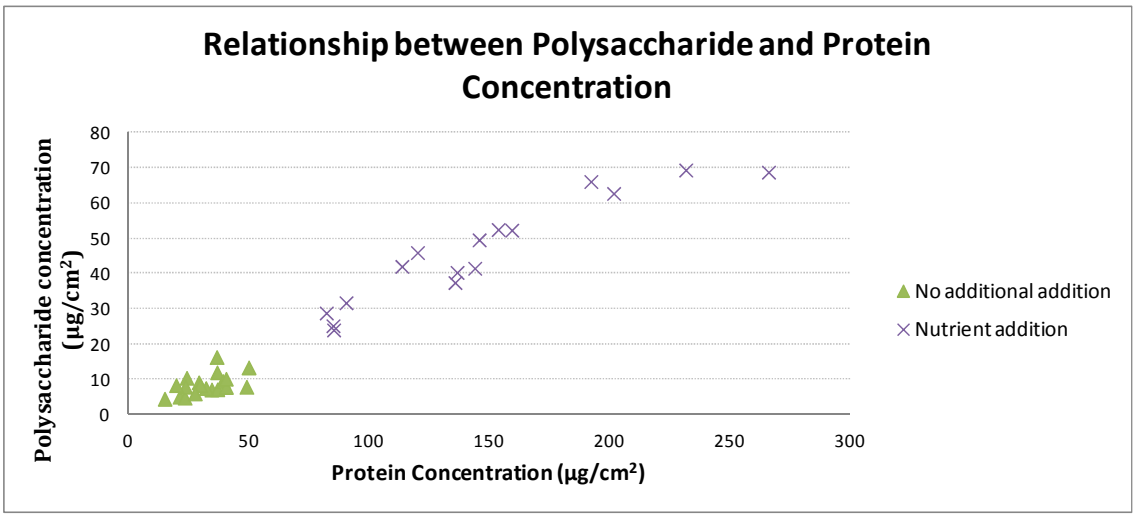


Figure 4-30: Relationship between polysaccharide and protein concentration

A very significant relationship ($p=2.23x10^{-24}$, $R^2=0.958$) was found between the measured protein and polysaccharide concentration for all the recorded data. This means that an accurate prediction can be obtained for protein or polysaccharide concentration if only the other one is measured. This relation was expected to some extent, since both these two parameters correlated well with the normalised flux decline.

4.5.4 Quantitative biofouling analysis summary

Different parameters were investigated to quantify biological fouling encountered on the membranes and link biological parameters to measured plant performance better. A significant increase was found in biological activity when a biodegradable compound was dosed in the feed tank. This can be used as a possible early warning system for biological fouling by measuring the amount of biodegradable components in the feed water. Future work is however required.

A highly significant relationship was also found between the measured protein and polysaccharide concentration with regard to the normalised flux decline in the presence of additional nutrients. A significant, but not well-correlated relationship was further found between the CFU and normalised flux decline, with no significant relationship found between the cell counts and flux decline. Vrouwenvelder et al. (2008) equally found a significant relationship between the ATP concentration and pressure increase (which can be related to flux decline), but no relationship with protein concentration.

A significant and well-correlated relationship was also found between protein concentration and polysaccharide concentration. This means that possibly only one of the parameter needs to be measured and the other one then determined by using inference. A significant, but not well-correlated relationship was further found between CFU and cell counts.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

The purpose of this study was to gain a deeper understanding – when dosing a non-oxidising biocide, 2,2-dibromo-3-propionamide (DBNPA) – of the effect of the operating variables (*i.e.* DBNPA dosage, frequency and dosing duration) on membrane biofouling and to propose an optimal dosing strategy. This was coupled with the determination of biological parameters (extracellular polymeric substances (EPS) content, cell count and plate count) that could be linked to system performance (membrane flux), thereby quantifying biological fouling better.

DBNPA was effective in reducing the amount of biofouling. Lower biological parameter concentrations were measured during all the runs (compared to blocks without any DBNPA dosing), regardless of the applied DBNPA dosing strategy. However, biofouling could be controlled best by a DBNPA dosage of 100 ppm for two hours, once per day. The total normalised flux then declined to only 85% of the initial value at the end of the run, compared to end-of-run declined normalised fluxes of around 60% (of the initial value) with the other dosing strategies. This observation supports the notion that a 'high enough' dosage should be applied for a 'long enough' period (*ca* two hours), instead of applying a high concentration shock-dosage for much shorter duration.

Protein and polysaccharide concentrations proved to be useful in quantifying biofouling. The system performance was directly influenced by the concentrations of these two parameters. A significant and reasonably correlated relationship was found between the protein concentration ($p=4.29 \times 10^{-5}$, $R^2=0.71$) and polysaccharide concentration ($p=0.0053$, $R^2=0.58$) with regard to the normalised flux decline when nutrients were dosed. A weaker, but still significant ($p=0.0011$, $R^2=0.54$) relationship was found between CFUs and normalised flux decline, with no significant ($p=0.14$) relationship between cell count and normalised flux decline. A strong and significant relationship ($p=2.23 \times 10^{-24}$, $R^2=0.958$) also existed between the polysaccharide and protein concentrations, with a notably weaker yet significant ($p=0.0025$, $R^2=0.25$) relationship between CFUs and cell count.

The following recommendations are made for future work:

- This study was limited to two cross-flow velocities (0.45 cm/s and 1.26 cm/s) at a specific pressure (7 bar). It is recommended that a wider range of velocities and pressures should be investigated to determine the possible hydrodynamic effect of biofouling combined with DBNPA dosing.
- This study was further limited to membrane sheets. Studies should be carried out on larger scale or with membrane block where a membrane sheet is on both sides of the feed channel represent spiral-wound elements more accurately.

- The run time of the experiments should be shortened to prevent the plateau formation, making it difficult to distinguish between dosing strategies. Lower-nutrient dosing limits the biological growth.

CHAPTER 6: REFERENCES

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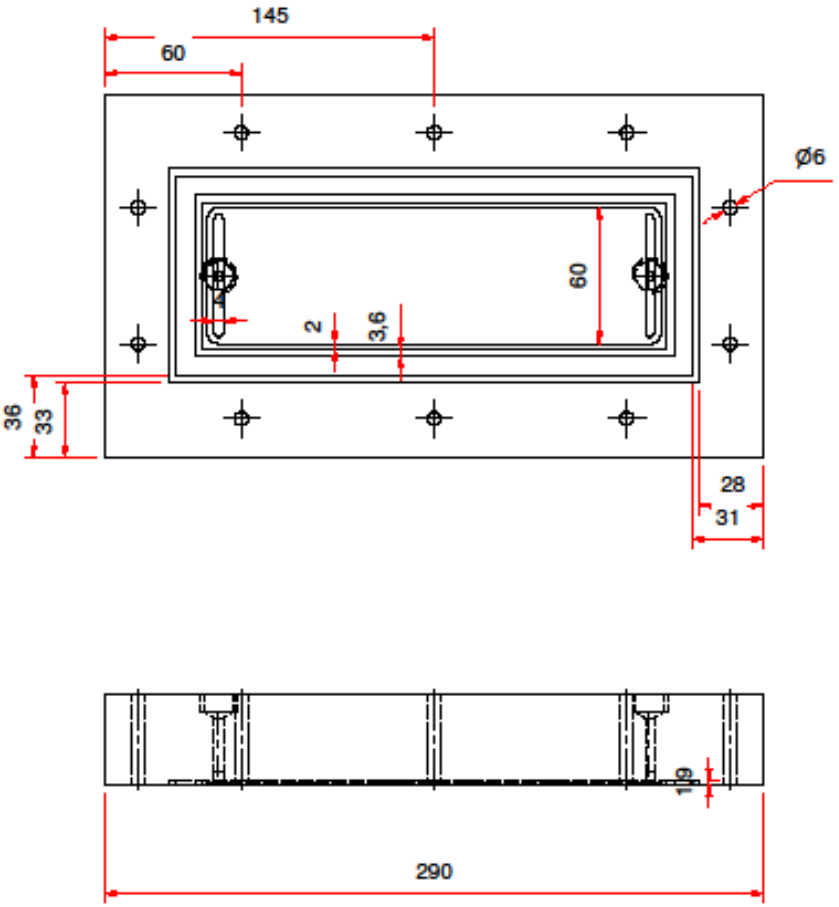
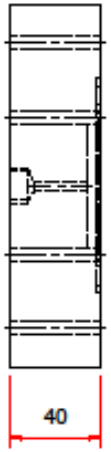
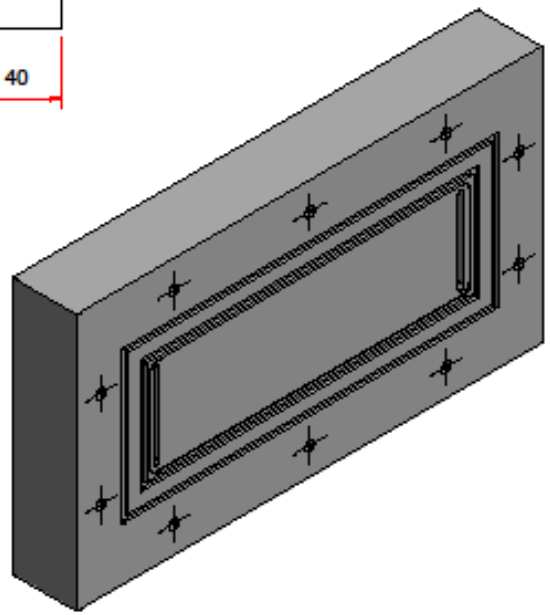
APPENDIX A: DETAILED BLOCK DESIGN







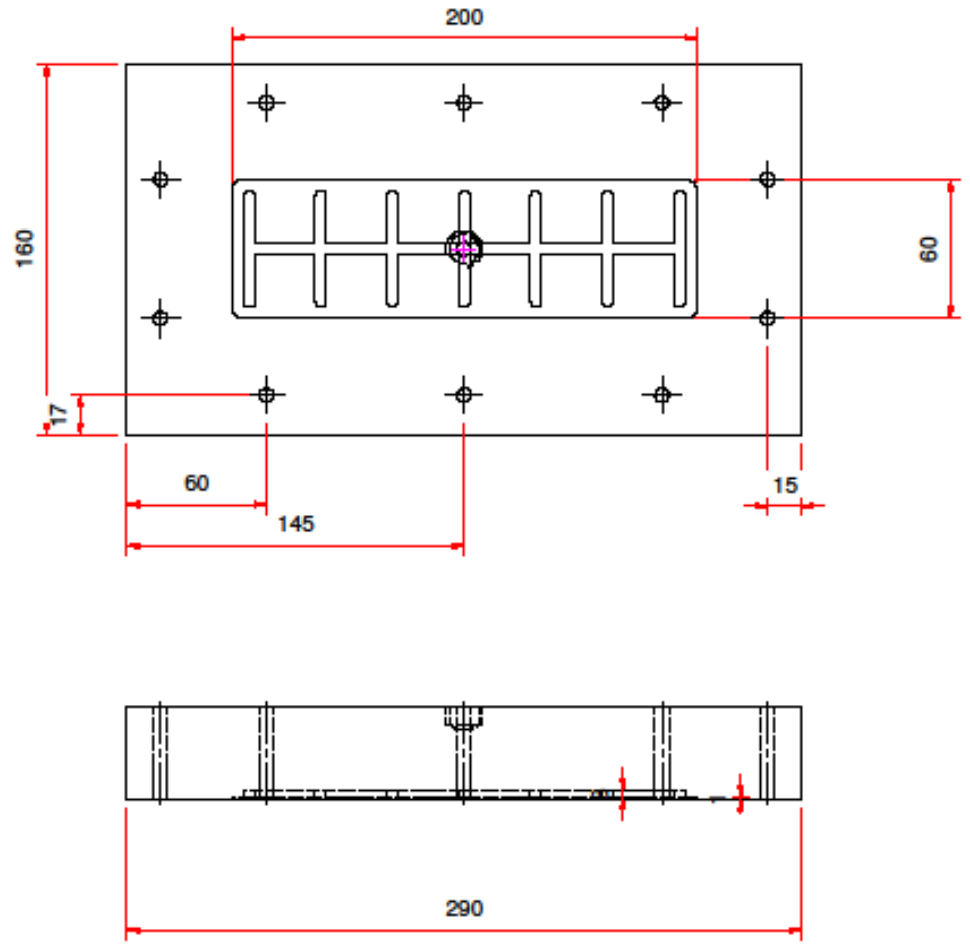
ITEM	BESKRYWING	AANTAL	MATERIAAL / SPESIFIKASIES

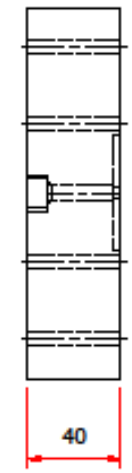
UNIVERSITEIT VAN STELLENBOSCH

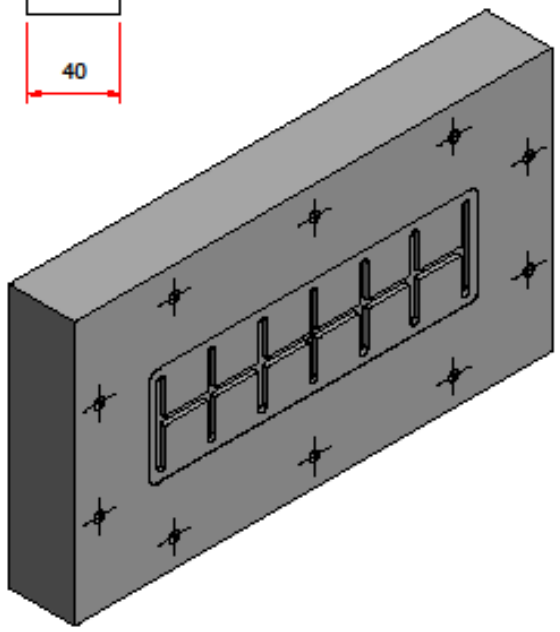
SKAAL OP A
MATE IN

TITEL: Feed Channel

STUDENTE No.	TEKENAAR	NAGESIEN	DATUM	VEL No. VAN VELLE	No.







ITEM	BESKRYWING	AANTAL	MATERIAAL / SPESIFIKASIES

UNIVERSITEIT VAN STELLENBOSCH

SKAAL OP A 1:2
MATE IN

TITEL: Permeate Side

STUDENTE No.	TEKENAAR	NAGESIEN	DATUM	VEL No. VAN VELLE	No.

APPENDIX B: FEED WATER ANALYSIS

A feed-water sample (after the cartridge and activated carbon filter) was send to the CSIR Analytical Laboratory in Stellenbosch for water analysis.

Table B-1: Composition of water sample

Sample Date	17 June 2014	
Analysis	**Unit**	**Value**
Calcium as Ca Dissolved	mg/l	7.0
Magnesium as Mg Dissolved	mg/l	0.7
Sulphate as SO_4 Dissolved	mg/l	7.6
Chloride as Cl	mg/l	10
Alkalinity as $CaCO_3$*	mg/l	11
Nitrate + Nitrite as N*	mg/l	<0.1
Total Organic Carbon (TOC)	mg/l	1.3
pH (Lab) (20°C)		7.2
Chemical Oxygen Demand*	mg/l	9

*Method not SANAS accredited and is not included in the SANAS Schedule of accreditation for the laboratory

APPENDIX C: CALIBRATION CURVES

Protein calibration curve

Bovine serum albumin (BSA) was used as standard for the calibration.

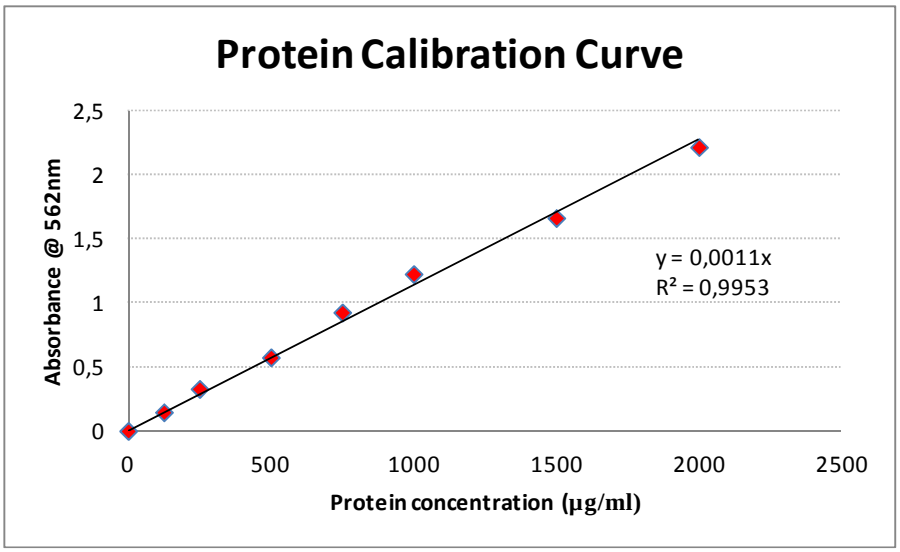


Figure C-1: Calibration curve for protein measurements

Table C-1: Data used for the calibration of the standard protein curve

Protein concentration (µg/ml)	Absorbance @562 nm
0	0
125	0.1458
250	0.3285
500	0.5748
750	0.9256
1000	1.226
1500	1.66425
2000	2.2184

Polysaccharide calibration curve

Glucose was used as standard for the calibration.

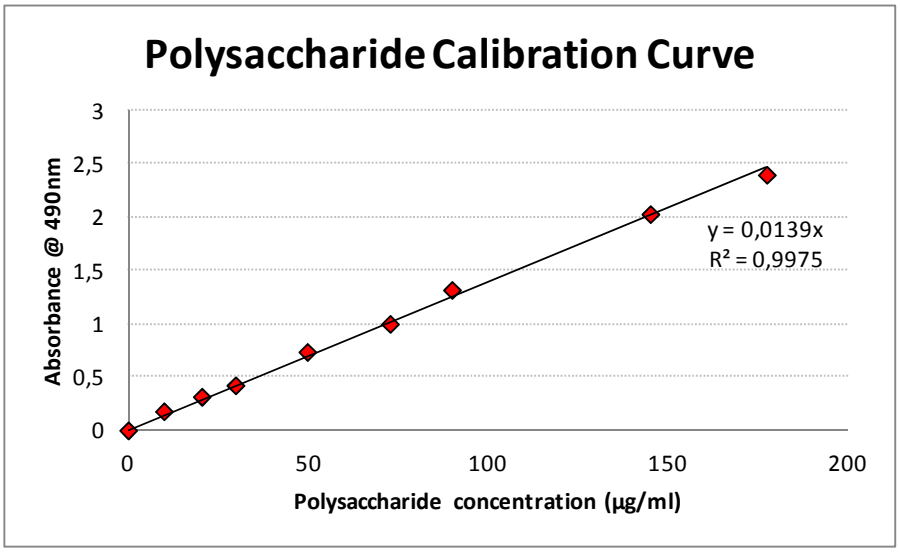


Figure C-2: Calibration curve for polysaccharide measurements

Table C-2: Data used for the calibration of the standard polysaccharide curve

Polysaccharide concentration (µg/ml)	Abs@490nm
0	0
9.92	0.1785
20.44	0.3158
29.82	0.4226
49.76	0.736
72.75	0.9977
89.94	1.3171
145.08	2.0284
177.41	2.3969

Flow meter calibration curve

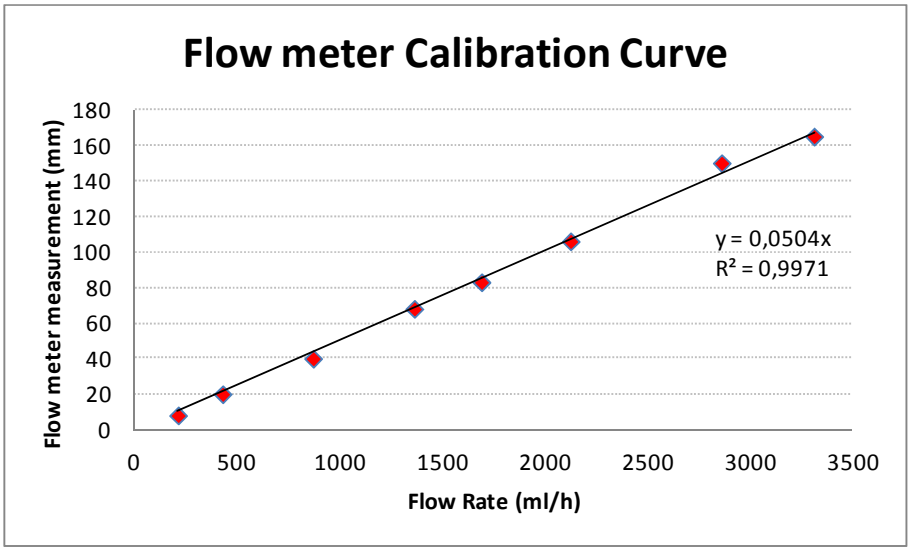


Figure C-3: Calibration curve for polysaccharide measurements

Table C-3: Data used for the calibration of the flow meters

Flow meter measurement (mm)	Flow rate (ml/h)
8	212.184
20	429.036
40	870.072
68	1362.96
83	1690.092
106	2124.576
150	2860.248
165	3311.028

APPENDIX D: EXPERIMENTAL RESULTS

D1: CFU after different biofilm removal and homogenisation techniques

Table D-1: Summary of different techniques used for biofilm removal from the membrane

Replicate	Removal Technique	Homogenisation	Sample Nr.
1	Scrape	B	1
		P	2
		B&P	3
	Scrape & Ultrasonic Bath	B	4
		P	5
		B&P	6
2	Scrape	B	7
		P	8
		B&P	9
	Scrape & Ultrasonic Bath	B	10
		P	11
		B&P	12
3	Scrape	B	13
		P	14
		B&P	15
	Scrape & Ultrasonic Bath	B	16
		P	17
		B&P	18

* B: Ultrasonic bath; P: Ultrasonic Probe

Table D-2: CFU after different methods used for the biofilm removal and homogenisation counted on NA and R_2A agar

NA (after 2 days) & R_2A (5days)								
Agar Used→	**NA**		**R_2A**		**NA**		**R_2A**	
Dilution→ / **Sample nr. ↓**	**10^3**	**10^4**	**10^4**	**Sample nr. ↓**	**10^3**	**10^4**	**10^3**	**10^4**
1	121	16	75	**10**	156	5		48
	111	8	52		107	9		93
2	196	18	104	**11**	73	24		87
	136	15	118		82	6		102
3	93	10	107	**12**	70	15		65
	170	11	119		53	9		84
4	90	14	90	**13**	55	8		45
	125	21	85		58	7		28
5	116	33	88	**14**	92	8		82
	175	30	97		65	15		68
6	210	39	163	**15**	109	11		69
	204	30	109		88	9		121
7	55	9	34	**16**	87	9	266	52
	40	8	89		88	10	-	80
8	110	5	65	**17**	107	9		79
	127	20	50		90	20		61
9	68	4	52	**18**	75	10		122
	27	6	52		73	dry		Contaminated

Table D-3: Increase in CFU for the different methods used for the biofilm removal and homogenisation on NA for an additional incubation time of 3 day

Increase in CFU after 5 days					
Agar→	**NA**			**NA**	
Dilution→ / **Sample nr. ↓**	**10^3**	**10^4**	**Sample nr. ↓**	**10^3**	**10^4**
1	9	1	**10**	8	2
	10	1		0	3
2	7	5	**11**	4	2
	5	2		5	1
3	16	15	**12**	8	1
	7	3		0	12
4	3	0	**13**	1	1
	6	4		12	2
5	14	3	**14**	15	0
	8	3		6	1
6	4	4	**15**	12	2
	14	2		9	2
7	1	0	**16**	16	2
	6	1		6	1
8	24	0	**17**	6	7
	13	4		7	0
9	5	6	**18**	9	0
	2	1		9	0

D2: Fouling runs data

Run 1: DI water

Table D-4: Flux, conductivity and temperature measurements for Run 1

Run 1		Block 1		Block 2		Block 3		Block 4		Feed		
	Dosing											
Date	Time (h)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Cond. (µS/cm)	Temp (°C)	Pressure (bar)
2014-06-17 15:22	0.0	12.7	13.89	4	15.32	3.5	15.73	2.3	15.58	8.9	15.4	7
2014-06-18 11:00	19.6	6.6	12.73	2.4	9.84	2.2	8.99	2.1	13.11	9.5	22.4	3
2014-06-18 14:00	22.6	0	12.55	0	8.66	0	9.86	0	10.83	7.2	22	3
2014-06-19 12:20	45.0	2.8	12.98	2.6	7.73	33.4	8.03	17.8	11.69	8.7	21.2	3
2014-06-19 15:00	47.6	19.3	13.25	3.6	9.39	2.6	9.01	2.6	11.48	10.1	21	3
2014-06-20 10:15	66.9	0	9.84	0	8.92	0	8.37	0	10.67	12.1	22.4	3
2014-06-20 16:00	72.6	11.8	11.98	4.4	9.52	2.4	8.68	2.8	10.38	5	20.1	3
2014-06-21 10:30	91.1	8.7	12.35	2.7	7.05	2	7.98	2.1	10.29	8.3	20.5	3
2014-06-22 21:30	126.1	0	7.98	0	7.48	0	7.94	0	8.76	10.4	21.1	3
2014-06-23 16:30	145.1	3.6	12.95	2.6	10.05	10.3	8.95	2.6	10.82	7.9	20.5	3
2014-06-24 10:30	163.1	9.3	12.84	1.7	8.07	4.8	8.99	1.5	10.40	9.8	20.1	3
2014-06-24 15:30	168.1	8.2	13.24	2.2	10.24	2.6	9.29	2.4	11.61	9.3	21.2	3
2014-06-25 10:30	187.1	4.6	12.64	3.2	9.21	2.7	8.85	2.2	10.43	10.4	20.8	3

Run 2

Table D-5: Flux, conductivity and temperature measurements for Run 2

		Block 1		Block 2		Block 3		Block 4		Feed	
	Cross flow	1.26 cm/s		0.45 cm/s		0.45 cm/s		1.26 cm/s		Cont DBNPA dosing	
Date	Time (h)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Cond. (µS/cm)	Temp(°C)
2014-06-27 15:00	0.0	7.6	15.94	6.3	14.52	5.4	15.86	7.1	20.38	96.2	18.5
2014-06-27 15:15	0.2	7.6	15.68	6.3	14.22	5.4	15.43	7.1	20.41	95.6	18.2
2014-06-27 15:30	0.5	7.6	15.94	6.3	15.16	5.4	16.28	7.1	21.74	95.6	18.2
2014-06-28 15:30	24.5	6.6	16.23	5.7	14.02	6.3	14.81	7.8	22.03	81.1	21.5
2014-06-29 14:00	47.0	11.9	14.54	4.9	11.90	3.8	12.91	1.9	21.77	62	21.5
2014-06-29 14:15	47.3	11.9	16.71	4.9	13.87	3.8	13.30	1.9	23.58	62	21.5
2014-06-30 12:00	69.0	11.9	16.08	4.9	13.99	3.8	14.24	1.9	22.42	62.5	21.5
2014-06-30 16:00	73.0	9.9	17.19	3.2	14.77	2.4	15.46	3.1	23.75	62.3	22.3
2014-06-30 16:00	73.0	2.7	17.41	3.3	15.40	3.6	14.85	7.2	23.68	62.3	22.3
2014-07-01 10:30	91.5	2.7	17.08	3.3	16.32	3.6	15.21	7.2	26.58	63.5	22
2014-07-01 11:00	92.0	4.8	17.48	4.9	15.07	3.2	14.34	4.1	24.32	63.5	22
2014-07-01 14:30	95.5	4.8	18.21	4.9	15.63	3.2	14.80	4.1	24.17	60.6	22.1
2014-07-02 11:00	116.0	3.5	16.97	4.1	13.69	3.4	13.32	3.5	23.45	68.2	22.5
2014-07-02 11:30	116.5	5.2	17.82	5	16.49	5.4	12.36	5.1	24.28	68.2	22.5
2014-07-02 13:30	118.5	5.2	17.68	5	14.62	5.4	14.52	5.1	24.95	61.8	22.1
2014-07-03 11:30	140.5	5.2	15.90	5	13.41	5.4	12.87	4	22.00	62.3	23.2
2014-07-03 11:30	140.5	6	17.05	5.5	13.07	6.3	13.86	4	22.41	62.3	23.2
2014-07-03 15:50	144.8	6	17.01	5.5	13.93	6.3	13.73	4	21.58	85.5	21.7
2014-07-04 10:30	163.5	3.3	16.21	4.1	12.17	5.5	12.38	8.6	21.50	68	22.3
2014-07-04 11:00	164.0	10.6	16.51	15.8	12.54	10.5	12.45	8.6	21.75	68	22.3

Table D-6: Protein and Polysaccharide measurements for Run 2

Proteins	Abs@562nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)
Zero	0.1128				
Block 1	0.2267	0.1139	100.15	33.38	34.64
	0.2291	0.1163	102.26	34.09	
	0.2372	0.1244	109.38	36.46	
Block 2	0.1845	0.0717	63.04	21.01	21.37
	0.1865	0.0737	64.80	21.60	
	0.1861	0.0733	64.45	21.48	
Block 3	0.1905	0.0777	68.32	22.77	23.53
	0.1953	0.0825	72.54	24.18	
	0.1935	0.0807	70.95	23.65	
Block 4	0.2325	0.1197	105.25	35.08	37.06
	0.2222	0.1094	96.19	32.06	
	0.2631	0.1503	132.15	44.05	

Polysaccharides	Abs@490nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)
Zero	0.0401				
Block 1	0.306	0.2659	19.15	6.38	7.23
	0.321	0.2809	20.23	6.74	
	0.3965	0.3564	25.67	8.56	
Block 2	0.2699	0.2298	16.55	5.52	5.20
	0.2255	0.1854	13.35	4.45	
	0.2744	0.2343	16.87	5.62	
Block 3	0.3337	0.2936	21.14	7.05	4.91
	0.1609	0.1208	8.70	2.90	
	0.2387	0.1986	14.30	4.77	
Block 4	0.2956	0.2555	18.40	6.13	7.28
	0.4236	0.3835	27.62	9.21	
	0.3115	0.2714	19.55	6.52	

Table D-7: CFU counts for Run 2

Dilution	10^3				Average CFU count	Average CFU/cm^2
Block 1 (high flow)	32	37	39		35.60	1.187E+05
	24	46				
Block 2 (low flow)	54	80			73.00	2.433E+05
	84	74				
Block 3 (low flow)	28	17			21.33	7.111E+04
	19					
Block 4 (high flow)	23	22			23.00	7.667E+04
	24					

Table D-8: Cell counts for Run 2

Dilution	10^1										
	Membrane										
	Block 1			**Block 2**			**Block 3**			**Block 4**	
	1	2	3	2	2	2	2	3	2	3	3	4
	1	1	2	1	3	1	2	3	2	4	4	4
	3	1	2	1	2	2	2	3	1	6	3	3
	2	2	1	3	4	2	3	5	4	3	2	2
	5	5	2	3	3	1	4	3	0	3	2	2
	4	3	2	4	5	5	4	4	3	2	4	5
	5	3	4	2	3	3	5	3	2	4	3	3
	2	6	3	4	3	3	4	5	1	4	5	2
	6	3	4	1	5	2	5	2	2	5	5	3
	5	2	1	4	1	2	2	0	1	4	3	2
	3	3	1	2	2	2	3	2	4	4	6	6
	2	2	3	2	4	3	1	7	5	3	6	3
	1	2	3	4	3	3	3	4	2	3	3	2
	6	4	2	2	1	1	1	3	2	1	4	4
	3	4	1	0	3	2	5	4	5	3	3	4
	4	5	5	5	2	5	3	4	2	4	3	4
	3	3	2	1	3	3	6	4	3	3	4	3
	2	3	3	1	4	3	3	3	3	5	5	3
	4	1	4	2	4	3	5	2	2	6	4	3
	3	4	4	3	4	5	2	1	2	2	4	3
Average per square	2.933			2.683			2.967			3.550		

Cell count (cells/µl)	1.173E+06	1.073E+06	1.187E+06	1.420E+06
Cell count (cells/cm^2)	3.911E+08	3.578E+08	3.956E+08	4.733E+08

Run 3

Table D-9: Flux, conductivity and temperature measurements for Run 3

		Block 1		Block 2		Block 3		Block 4		Feed	
	Dosing	200 ppm, 15 min, daily		200 ppm, 30 min, daily		100 ppm, 15 min, daily		Control		Tap water	
Date	Time (h)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Cond. (µS/cm)	Temp(°C)
2014-07-25 13:00	0.0	13.8	9.55	5.5	13.72	4.1	5.39	3.8	7.79	69.8	16.5
2014-07-25 13:00	0.0	13.8	10.17	5.5	13.98	4.1	5.88	3.8	7.81	69.8	16.5
2014-07-25 13:00	0.0	13.8	10.22	5.5	14.35	4.1	5.58	3.8	8.40	69.8	16.5
2014-07-26 13:00	24.0	13.8	10.69	5.5	11.68	4.1	6.23	3.8	8.34	71.2	22.1
2014-07-26 13:00	24.0	13.8	10.97	5.5	13.06	4.1	5.91	3.8	8.54	71.2	22.1
2014-07-27 14:50	49.8	2.2	10.52	3.4	12.43	5.4	6.41	8.8	7.77	62.5	21.7
2014-07-27 14:50	49.8	2.2	11.06	3.4	13.05	5.4	6.51	8.8	8.54	62.5	21.7
2014-07-28 10:00	69.0	2	10.46	3.3	11.19	9.5	6.33	2.2	7.24	63.5	21.4
2014-07-28 10:00	69.0	2	10.81	3.3	11.67	9.5	6.50	2.2	8.28	63.5	21.4
2014-07-28 16:00	75.0	10.9	10.82	3	12.50	4.6	6.61	4.9	8.99	66	21.8
2014-07-28 16:00	75.0	10.9	11.10	3	12.63	4	6.50	4.9	9.03	66	21.8
2014-07-29 10:00	93.0	2.2	12.09	2.1	10.83	4	6.44	4.4	8.71	61.6	21.4
2014-07-29 10:00	93.0	8.6	11.20	2.1	12.50	3.1	6.59	4.4	8.71	61.6	21.4
2014-07-29 16:00	99.0	8.6	11.66	2.6	13.38	3.1	5.43	4.4	8.87	61.7	22
2014-07-29 16:00	99.0	3.3	12.09	2.6	13.98	3.6	7.03	2.6	9.04	61.7	22
2014-07-30 10:00	117.0	3.3	11.24	3.3	11.70	3.6	6.57	2.6	9.00	62.6	21.2
2014-07-30 10:00	117.0	2.6	11.39	3.3	11.81	3.4	6.57	3.6	9.16	62.6	21.2
2014-07-30 16:00	123.0	2.6	12.08	2.9	13.24	3.4	6.75	3.6	9.46	66.3	22.1
2014-07-30 16:00	123.0	6.7	11.94	2.9	13.36	4.1	6.99	3.2	9.44	66.3	22
2014-07-31 10:00	141.0	6.7	17.42	4.9	15.71	4.1	10.51	3.2	12.67	69.9	22.2
2014-07-31 10:00	141.0	5.5	10.93	4.9	10.22	5	6.52	4.4	8.18	69.9	22.2
2014-07-31 16:00	147.0	5.5	11.26	3	11.13	4	6.90	4.4	9.08	68.8	22.5
2014-07-31 16:00	147.0	6.7	10.97	3	11.00	2.6	6.90	4.1	8.81	68.8	22.5
2014-08-01 08:00	163.0	6.7	10.59	2.6	10.46	2.6	6.19	4.1	8.42	70.1	21.7
2014-08-01 08:00	163.0	6.7	11.08	2.6	11.29	3	6.54	2.3	7.87	70.1	21.7

Table D-10: Protein and Polysaccharide measurements for Run 3

	Proteins	Abs@562nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)		Polysaccharides	Abs@490nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)
	Zero	0.3581						**Zero**	0.0134				
Membrane	200 ppm, 15 min, daily	0.4439	0.0858	75.44	25.15	29.32	**Membrane**	200 ppm, 15 min, daily	0.3871	0.3737	26.91	8.97	9.28
		0.4611	0.103	90.56	30.19				0.3844	0.371	26.72	8.91	
		0.4694	0.1113	97.86	32.62				0.4288	0.4154	29.92	9.97	
	200 ppm, 30 min, daily	0.4681	0.11	96.72	32.24	32.33		200 ppm, 30 min, daily	0.4243	0.4109	29.59	9.86	7.62
		0.4751	0.117	102.87	34.29				0.3329	0.3195	23.01	7.67	
		0.462	0.1039	91.35	30.45				0.2353	0.2219	15.98	5.33	
	100 ppm, 15 min, daily	0.4131	0.055	48.36	16.12	15.20		100 ppm, 15 min, daily	0.2446	0.2312	16.65	5.55	4.61
		0.4154	0.0573	50.38	16.79				0.2384	0.225	16.20	5.40	
		0.4014	0.0433	38.07	12.69				0.1336	0.1202	8.66	2.89	
	Control	0.4344	0.0763	67.09	22.36	24.32		Control	0.4169	0.4035	29.06	9.69	10.53
		0.4334	0.0753	66.21	22.07				0.4083	0.3949	28.44	9.48	
		0.4554	0.0973	85.55	28.52				0.5304	0.517	37.23	12.41	
Spacer	200 ppm, 15 min, daily	0.3822	0.0241	21.19	3.53	2.48	**Spacer**	200 ppm, 15 min, daily	0.04288	0.02948	2.12	0.35	0.29
		0.3679	0.0098	8.62	1.44				0.0329	0.0195	1.40	0.23	
	200 ppm, 30 min, daily	0.4453	0.0872	76.67	12.78	10.29		200 ppm, 30 min, daily	0.0343	0.0209	1.51	0.25	0.40
		0.4114	0.0533	46.86	7.81				0.059	0.0456	3.28	0.55	
	100 ppm, 15 min, daily	0.3903	0.0322	28.31	4.72	3.37		100 ppm, 15 min, daily	0.1124	0.099	7.13	1.19	1.01
		0.3719	0.0138	12.13	2.02				0.0833	0.0699	5.03	0.84	
	Control	0.4099	0.0518	45.54	7.59	5.22		Control	0.0681	0.0547	3.94	0.66	0.51
		0.3775	0.0194	17.06	2.84				0.0431	0.0297	2.14	0.36	

Table D-11: CFU counts for Run 3

	Dilution	10^3			10^4				Average count	Average CFU/cm^2
Membrane	200 ppm, 15 min, daily	82	84	98					88.00	2.933E+05
	200 ppm, 30 min, daily	37	67	65					56.33	1.878E+05
	100 ppm, 15 min, daily	42	53	36					43.67	1.456E+05
	Control		22		193	220	260		224.33	7.478E+06
		24								
	Dilution	10^1								
Spacer	200 ppm, 15 min, daily	74	73						73.50	1.225E+03
	200 ppm, 30 min, daily	83	86						84.50	1.408E+03
	100 ppm, 15 min, daily	129	130						129.50	2.158E+03
	Control	TNTC	TNTC						-	-

Table D-12: Cell counts for Run 3

Dilution	10^1										10^0			
Membrane											**Spacer**			
200 ppm, 15 min, daily			**200 ppm, 30 min, daily**			**100 ppm, 15 min, daily**			**Control**		**200 ppm, 15 min, daily**	**200 ppm, 30 min, daily**	**100 ppm, 15 min, daily**	**Control**
4	2	3	2	2	3	1	2	3	3	3	1	3	2	1	2
2	3	2	3	2	2	3	1	2	2	4	1	3	1	3	3
0	1	3	2	2	1	0	1	1	3	1	2	0	1	1	2
3	2	0	1	2	3	1	2	1	5	4	3	1	0	0	0
2	2	2	4	3	1	2	1	3	1	4	2	3	3	0	0
4	3	3	3	2	1	2	0	2	2	3	5	4	1	0	0
2	4	3	1	2	3	0	1	2	1	2	1	1	3	1	1
2	7	1	3	2	1	3	2	1	5	5	2	2	2	2	2
2	3	4	1	1	2	1	2	1	2	2	2	2	1	2	1
1	3	2	2	0	4	2	0	1	3	1	2	2	2	3	1
3	1	3	3	1	2	3	3	2	5	4	1	1	1	0	2
3	1	2	3	4	2	1	1	5	2	0	4	3	0	1	1
1	1	5	2	0	1	1	1	4	4	2	5	0	3	1	2
4	4	1	1	3	4	2	1	3	2	3	4	4	2	4	2
0	3	2	1	6	1	1	1	2	2	2	3	0	0	3	1
4	3	5	2	2	1	2	0	3	3	4	1	4	1	1	1
2	3	3	2	3	1	3	3	3	0	4	2	3	2	1	3
2	0	3	2	1	4	1	1	1	0	3	2	1	2	0	5
0	3	2	0	1	4	3	1	1	2	3	2	1	1	0	4

	1	3	1	0	3	1	4	0	0	4	1	1	1	0	2	1
Average per square	2.40E+00			2.03E+00			1.68E+00			2.53E+00			2.0E+00	1.4E+00	1.3E+00	1.7E+00
Cell count(cells/µl)	9.60E+05			8.13E+05			6.73E+05			1.01E+06			7.8E+04	5.6E+04	5.2E+04	6.8E+04
Cell count(cells/cm^2)	3.20E+08			2.71E+08			2.24E+08			3.38E+08			2.6E+07	1.9E+07	1.7E+07	2.3E+07

Run 4

Table D-13: Flux, conductivity and temperature measurements for Run 4

		Block 1		Block 2		Block 3		Block 4		Feed	
	Dosing	10 ppm, 2 h, daily		100 ppm, 30 min, daily		10 ppm, 30 min, daily		Control		Tap water	
Date	Time (h)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Cond. (µS/cm)	Temp(°C)
2014-08-19 13:40	0.0	6.1	12.98	3.7	16.07	4	8.80	3.4	13.54	75.9	20.5
2014-08-19 13:40	0.0	6.1	13.69	3.7	16.96	4	9.29	3.4	14.12	75.9	20.5
2014-08-19 13:40	0.0	6.1	13.63	3.7	17.10	4	9.29	3.4	13.51	75.9	20.5
2014-08-20 13:40	24.0	5.2	14.56	6.4	16.67	5.1	7.63	4.6	15.13	73.2	22
2014-08-20 16:00	26.3	5.2	13.37	6.4	15.65	5.1	9.00	4.6	13.56	73.2	22
2014-08-20 16:00	26.3	5.2	13.65	6.4	15.99	5.1	8.90	4.6	13.37	76.7	21.4
2014-08-21 08:30	42.8	7	12.68	5.8	14.97	6.7	8.20	5.7	12.01	75.6	21.1
2014-08-21 08:30	42.8	7.3	13.14	5.8	15.02	6.7	8.27	5.7	11.83	75.6	21.1
2014-08-21 16:00	50.3	8.7	13.77	4.3	16.14	3.4	9.00	3.3	13.87	75.7	21.1
2014-08-21 16:00	50.3	8.7	13.59	4.63	15.85	3.4	9.00	3.3	13.50	75.7	21.1
2014-08-22 09:30	67.8	4.2	12.92	3.3	15.25	3.4	8.68	3.9	13.03	70	21.4
2014-08-22 09:30	67.8	4.2	13.23	3.3	15.72	3.4	9.14	3.9	13.54	70	21.4
2014-08-22 16:00	74.3	4.9	13.73	6.8	15.74	4.5	8.93	4.1	13.36	60	21.2
2014-08-22 16:00	74.3	4.9	13.52	6.8	15.92	4.5	9.10	4.1	13.26	60	21.2
2014-08-23 09:30	91.8	4.8	11.90	3.2	13.70	4.1	7.49	3.3	11.30	64.2	20.4
2014-08-23 09:30	91.8	4.8	11.78	3.2	13.69	4.1	7.56	3.3	11.08	64.2	20.4
2014-08-24 15:30	121.8	6.6	11.27	7.5	11.84	4.6	7.33	5.3	10.32	68.2	20.4
2014-08-24 15:30	121.8	6.6	11.59	7.5	12.32	4.6	7.69	5.3	10.61	68.2	20.4
2014-08-25 09:00	139.3	11.3	11.38	5	12.90	4.4	7.81	3.9	11.50	80.3	19.7
2014-08-25 09:00	139.3	11.3	11.35	5	13.16	4.4	7.97	3.9	11.56	80.3	19.7
2014-08-25 16:00	146.3	5.7	12.39	6.9	14.21	4.8	8.29	5.4	12.44	75.9	20.9
2014-08-25 16:00	146.3	5.7	12.26	6.9	14.43	4.8	8.51	5.4	12.41	75.9	20.9
2014-08-26 09:00	163.3	4.7	11.66	3	13.32	3.5	7.76	3.3	11.68	68.9	21.5
2014-08-26 09:00	163.3	4.7	11.75	3	13.79	3.5	8.03	3.3	12.02	68.9	21.5

Table D-14: Protein and Polysaccharide measurements for Run 4

	Proteins	Abs@562nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)		Polysaccharides	Abs@490nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)
	Zero	0.0855						**Zero**	0.0213				
Membrane	10 ppm, 2 h, daily	0.2349	0.2349	206.53	68.84	74.24	**Membrane**	10 ppm, 2 h, daily					7.99
		0.2685	0.2685	236.08	78.69				0.5241	0.5028	36.21	12.07	
		0.2565	0.2565	225.53	75.18				0.5169	0.4956	35.69	11.90	
	100 ppm, 30 min, daily	0.2571	0.2571	226.05	75.35	75.13		100 ppm, 30 min, daily	0.5578	0.5365	38.64	12.88	13.47
		0.2721	0.2721	239.24	79.75				0.6044	0.5831	41.99	14.00	
		0.2398	0.2398	210.84	70.28				0.5853	0.564	40.62	13.54	
	10 ppm, 30 min, daily	0.1864	0.1864	163.89	54.63	55.01		10 ppm, 30 min, daily	0.3562	0.3349	24.12	8.04	8.49
		0.1919	0.1919	168.73	56.24				0.3904	0.3691	26.58	8.86	
		0.1848	0.1848	162.48	54.16				0.3784	0.3571	25.72	8.57	
	Control	0.1882	0.1882	165.47	55.16	61.84		Control	0.6655	0.6442	46.39	15.46	16.44
		0.2267	0.2267	199.32	66.44				0.6635	0.6422	46.25	15.42	
		0.2181	0.2181	191.76	63.92				0.7895	0.7682	55.32	18.44	
Spacer	10 ppm, 2 h, daily	0.0944	0.0944	83.00	13.83	20.08	**Spacer**	10 ppm, 2 h, daily	0.0441	0.0228	1.64	0.27	0.27
		0.1797	0.1797	158.00	26.33				0.6541	0.6328	45.57	7.60	
	100 ppm, 30 min, daily	0.1454	0.1454	127.84	21.31	19.34		100 ppm, 30 min, daily	0.0383	0.017	1.22	0.20	0.17
		0.1186	0.1186	104.28	17.38				0.0328	0.0115	0.83	0.14	
	10 ppm, 30 min, daily	0.0953	0.0953	83.79	13.97	142.83		10 ppm, 30 min, daily	0.0374	0.0161	1.16	0.19	0.43
		1.8541	1.8541	1630.20	271.70				0.077	0.0557	4.01	0.67	
	Control	0.194	0.194	170.57	28.43	21.09		Control	0.0732	0.0519	3.74	0.62	0.88
		0.0939	0.0939	82.56	13.76				0.1165	0.0952	6.86	1.14	

Table D-15: CFU counts for Run 4

	Dilution	10^3			10^4				**Average count**	**Average CFU/cm^2**
Membrane	10 ppm, 2 h, daily	93	68	69					76.67	2.556E+05
	100 ppm, 30 min, daily	15	41						28	9.333E+04
	10 ppm, 30 min, daily	42	4						23	7.667E+04
	Control				150	104	158		137.33	4.578E+06
	Dilution	10^1		10^2						
Spacer	10 ppm, 2 h, daily	222	175	37	30				198.5	3.308E+03
	100 ppm, 30 min, daily	55	49						52	8.667E+02
	10 ppm, 30 min, daily	173	166						169.5	2.825E+03
	Control			154	156				155	2.583E+03

Table D-16: Cell counts for Run 4

	Dilution	10^1											10^0			
	Membrane												**Spacer**			
	10 ppm, 2 h, daily			**100 ppm, 30 min, daily**			**10 ppm, 30 min, daily**			**Control**			**10 ppm, 2 h, daily**	**100 ppm, 30 min, daily**	**10 ppm, 30 min, daily**	**Control**
	1	4	4	1	4	1	1	3	3	5	3	1	1	1	1	1
	4	9	3	4	1	1	3	3	0	7	6	2	1	1	0	1
	0	8	3	3	1	0	1	2	2	2	3	1	0	0	0	0
	5	8	1	2	0	2	0	0	1	3	3	2	1	0	1	1
	5	7	1	5	2	0	0	3	2	1	4	1	2	1	0	1
	2	3	2	4	6	2	4	2	1	3	3	5	2	1	3	0
	1	3	3	3	4	1	2	1	3	1	3	4	1	1	2	0
	4	3	2	2	1	1	3	1	1	2	4	1	1	0	1	1
	5	2	2	1	2	0	2	2	0	3	3	1	1	1	1	3
	1	3	1	3	2	2	2	6	1	5	3	3	0	2	0	1
	3	4	1	0	3	2	3	1	2	0	2	3	1	1	1	1
	3	13	2	1	2	4	2	5	0	3	2	6	1	1	1	0
	1	3	1	1	3	1	1	3	5	4	2	3	2	1	1	1
	1	7	4	4	3	1	1	4	0	3	3	2	1	1	2	3
	2	8	1	1	0	3	1	3	4	3	4	3	1	0	0	0
	1	6	1	3	4	3	1	4	2	6	1	3	1	0	0	1
	2	7	3	2	2	6	0	6	5	0	0	2	0	1	1	1
	0	8	2	4	1	1	2	2	1	3	4	0	0	1	1	0
	0	4	1	1	5	2	0	4	0	1	1	1	1	0	1	0
	1	4	1	2	1	3	2	2	1	2	2	4	1	1	0	0
Average per square	3.25E+00			2.17E+00			2.03E+00			2.68E+00			9.5E-01	7.5E-01	8.5E-01	8.0E-01
Cell count(cells/µl)	1.30E+06			8.67E+05			8.13E+05			1.07E+06			3.8E+04	3.0E+04	3.4E+04	3.2E+04
Cell count(cells/cm^2)	4.33E+08			2.89E+08			2.71E+08			3.58E+08			1.3E+07	1.0E+07	1.1E+07	1.1E+07

Run 5

Table D-17: Flux, conductivity and temperature measurements for Run 5

		Block 1		Block 2		Block 3		Block 4		Feed	
	Dosing	10 ppm, 2 h, daily		Control		100 ppm, 30 min, every 2nd day		Dead End		Tap water	
Date	Time (h)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Cond. (µS/cm)	Temp(°C)
2014-08-28 13:00	0.0	19.6	10.77	3.8	12.05	2.8	7.75	2.1	10.73	69.6	17.2
2014-08-28 13:00	0.0	19.6	10.89	3.8	12.17	2.8	7.73	2.1	10.63	69.6	17.2
2014-08-28 13:00	0.0	19.6	11.29	3.8	12.92	2.8	7.97	2.1	11.18	69.6	17.2
2014-08-29 09:30	20.5	4.4	11.93	3.5	13.68	5.7	8.45	11.6	8.50	71.5	19
2014-08-29 09:30	20.5	4.4	10.73	3.5	13.67	5.7	8.68	11.6	8.17	71.5	19
2014-08-29 15:00	26.0	3.6	12.55	3	11.94	3.4	8.91	13.8	8.07	72	19.7
2014-08-29 15:00	26.0	3.6	13.45	3	14.22	3.4	9.54	13.8	2.98	72	19.7
2014-08-30 10:30	45.5	7.3	12.14	6.4	10.57	7.1	10.17	20.3	4.13	64.8	19.8
2014-08-30 10:30	45.5	7.3	12.34	6.4	14.19	7.1	8.41	20.3	9.52	64.8	19.8
2014-08-31 18:00	77.0	6.6	10.78	9.3	11.39	14.2	8.89	27.6	7.60	70	22.3
2014-08-31 18:00	77.0	6.6	10.05	9.3	12.90	14.2	7.55	27.6	8.98	70	22.3
2014-09-01 10:00	93.0	3.2	11.27	4	12.12	6.1	8.28	41.2	7.59	68.6	20.2
2014-09-01 10:00	93.0	3.2	11.25	4	10.60	6.1	9.00	41.2	7.01	68.6	20.2
2014-09-01 15:30	98.5	3.3	12.50	4.1	14.02	7.7	10.78	41.4	8.56	66.4	23.5
2014-09-01 15:30	98.5	3.3	10.70	4.1	14.24	7.7	8.48	41.4	8.85	66.4	23.5
2014-09-02 09:30	116.5	4.5	10.98	4.4	12.70	3.5	8.92	37.7	7.95	69.2	21.8
2014-09-02 09:30	116.5	4.5	11.24	4.4	11.35	3.5	10.34	37.7	8.04	69.2	21.8
2014-09-02 16:00	123.0	6.7	10.04	3.1	13.59	3.1	8.29	38.4	8.04	60.2	22.1
2014-09-02 16:00	123.0	6.7	11.85	3.1	13.05	3.1	10.83	38.4	8.53	60.2	22.1
2014-09-03 09:00	140.0	6.8	9.93	4.6	8.60	3.8	8.45	38.4	6.89	62	20.4
2014-09-03 09:00	140.0	6.8	9.88	4.6	11.20	3.8	8.42	38.4	6.87	62	20.4
2014-09-03 16:00	147.0	8.6	9.55	4.3	9.22	6.3	8.83	38.9	5.71	65.8	21
2014-09-03 16:00	147.0	8.6	10.69	4.3	12.06	6.3	8.56	38.9	7.21	65.8	21
2014-09-04 09:00	164.0	8.4	9.15	5.4	10.92	20.4	8.49	42.1	5.86	66.1	21.1
2014-09-04 09:00	164.0	8.4	9.38	5.4	10.19	20.4	5.44	42.1	5.31	66.1	21.1

Table D-18: Protein and Polysaccharide measurements for Run 5

	Proteins	Abs@562nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)		Polysaccharides	Abs@490nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)
	Zero	0.10385						**Zero**	0.02955				
Membrane	**10 ppm, 2 h, daily**	0.1733	0.1733	152.37	50.79	63.90	**Membrane**	**10 ppm, 2 h, daily**	0.3507	0.32115	23.13	7.71	8.03
		0.1966	0.1966	172.86	57.62				0.3875	0.35795	25.78	8.59	
		0.2842	0.2842	249.88	83.29				0.3535	0.32395	23.33	7.78	
	Control	0.2208	0.2208	194.14	64.71	67.43		**100 ppm, 30 min, daily**	0.5594	0.52985	38.16	12.72	12.10
		0.2453	0.2453	215.68	71.89				0.499	0.46945	33.81	11.27	
		0.2241	0.2241	197.04	65.68				0.543	0.51345	36.98	12.33	
	100 ppm, 30 min, every 2nd day	0.1752	0.1752	154.04	51.35	50.41		**10 ppm, 30 min, daily**	0.3301	0.30055	21.64	7.21	8.47
		0.1777	0.1777	156.24	52.08				0.3895	0.35995	25.92	8.64	
		0.1631	0.1631	143.40	47.80				0.4274	0.39785	28.65	9.55	
	Dead End	0.184	0.184	161.78	53.93	54.96		**Control**	0.4063	0.37675	27.13	9.04	9.71
		0.1992	0.1992	175.14	58.38				0.4593	0.42975	30.95	10.32	
		0.1794	0.1794	157.74	52.58				0.436	0.40645	29.27	9.76	
Spacer	**10 ppm, 2 h, daily**	0.1108	0.1108	97.42	16.24	17.13	**Spacer**	**10 ppm, 2 h, daily**	0.0853	0.05575	4.01	0.67	0.75
		0.123	0.123	108.15	18.02				0.0991	0.06955	5.01	0.83	
	Control	0.1027	0.1027	90.30	15.05	15.58		**100 ppm, 30 min, daily**	0.1046	0.07505	5.40	0.90	0.67
		0.1099	0.1099	96.63	16.10				0.0664	0.03685	2.65	0.44	
	100 ppm. 30 min every 2nd day	0.1136	0.1136	99.88	16.65	26.68		**10 ppm, 30 min, daily**	0.1981	0.16855	12.14	2.02	1.52
		0.2506	0.2506	220.34	36.72				0.1147	0.08515	6.13	1.02	
	Dead End	0.1119	0.1119	98.39	16.40	22.88		**Control**	0.0811	0.05155	3.71	0.62	0.73
		0.2004	0.2004	176.20	29.37				0.099	0.06945	5.00	0.83	

Table D-19: CFU counts for Run 5

	Dilution	10^2			10^3			10^4				Average count	Average CFU/cm^2
Membrane	10 ppm, 2 h, daily	11	5	30								14,00	4,67E+03
		10											
	100 ppm, 30 min, daily				14	13	12					6,27	2,09E+04
	10 ppm, 30 min, daily				8	7	5					2,50	8,33E+03
								12	10	11			
	Control											3,11	1,04E+05
	Dilution	10^1		10^2									
Spacer	10 ppm, 2 h, daily	95	250									172,50	2,88E+03
	100 ppm, 30 min, daily			45		3	4					38,33	6,39E+02
	10 ppm, 30 min, daily			11								11,00	1,83E+02
	Control	-	-	-								-	-

Table D-20: Cell counts for Run 5

Dilution	10^1												10^0			
	Membrane												**Spacer**			
	10 ppm, 2 h, daily			**Control**			**100 ppm, 30 min, every 2nd day**			**Dead End**			**10 ppm, 2 h, daily**	**Control**	**100 ppm, 30 min, every 2nd day**	**Dead End**
	0	2	2	3	1	1	1	2	1	4	3	3	1	1	2	2
	4	2	0	0	0	1	0	2	1	0	1	0	1	2	0	2
	1	1	0	3	1	2	1	5	0	0	1	1	2	1	1	4
	0	1	1	3	1	1	1	1	2	1	6	2	0	1	2	1
	2	0	1	2	2	1	1	1	3	0	2	5	1	2	1	1
	0	4	0	4	1	2	2	2	1	3	2	1	3	4	1	2
	1	4	0	2	1	1	1	1	1	1	0	1	3	1	2	4
	1	0	1	1	2	4	1	3	2	1	1	1	1	2	2	1
	2	0	1	3	3	1	2	0	1	3	2	2	1	1	1	0
	0	2	1	2	5	1	1	0	1	1	0	1	1	3	1	0
	0	1	4	4	1	0	0	2	0	2	1	1	0	1	1	0
	1	1	1	2	1	5	1	1	0	3	4	0	1	0	0	2
	2	1	2	1	1	3	0	2	1	4	1	1	0	1	1	1
	1	1	2	3	1	1	0	2	1	4	3	0	1	1	1	3
	1	2	3	3	2	2	1	0	2	1	2	1	0	3	2	2
	0	1	2	2	3	0	1	2	3	3	3	1	1	4	1	2
	3	2	1	1	2	4	1	1	2	1	1	2	1	2	1	1
	1	1	1	5	3	2	2	1	1	1	2	3	0	4	0	2
	0	1	1	2	2	2	3	2	1	1	1	2	0	2	1	1
	1	1	0	1	0	2	2	1	3	0	2	2	0	1	1	2
Average per square	1.22E+00			1.93E+00			1.33E+00			1.70E+00			9.0E-01	1.9E+00	1.1E+00	1.7E+00
Cell count(cells/µl)	4.87E+05			7.73E+05			5.33E+05			6.80E+05			3.6E+04	7.4E+04	4.4E+04	6.6E+04
Cell count(cells/cm^2)	1.62E+08			2.58E+08			1.78E+08			2.27E+08			1.2E+07	2.5E+07	1.5E+07	2.2E+07

Run 6

Table D-21: Flux, conductivity and temperature measurements for Run 6

		Block 1		Block 2		Block 3		Block 4		Feed	
	Dosing	10 ppm, 2 h, daily		100 ppm, 30 min, daily		10 ppm, 30 min, daily		Control		Tap water	
Date	Time (h)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Cond. (µS/cm)	Temp(°C)
2014-09-09 11:00	0.0	4.7	14.29	4.4	14.29	4.2	8.09	3	13.37	62.3	17.1
2014-09-09 11:00	0.0	4.7	12.98	4.1	14.52	4.2	7.69	3	12.91	62.3	17.2
2014-09-09 11:00	0.0	4.7	12.96	4.1	14.88	4.2	7.84	3	13.65	62.3	18.1
2014-09-09 16:00	5.0	12.4	16.66	5.1	17.27	4.8	8.89	4.3	16.38	61.3	21.2
2014-09-09 16:00	5.0	12.4	16.66	5.1	17.36	4.8	9.47	4.3	14.35	61.3	21.2
2014-09-10 08:30	21.5	21.9	14.41	6.5	13.02	6.2	9.92	5.5	10.36	62.5	21.1
2014-09-10 08:30	21.5	21.9	15.49	6.5	13.40	6.2	8.39	5.5	13.89	62.5	21.1
2014-09-10 16:00	29.0	6.1	15.31	4.6	14.05	4.4	8.86	4.1	14.03	61.1	21.4
2014-09-10 16:00	29.0	6.1	14.54	4.6	14.10	4.4	8.98	4.1	10.40	61.1	21.4
2014-09-11 09:00	46.0	6.4	13.48	5.2	11.28	4.8	8.36	4	13.32	60.5	20.1
2014-09-11 09:00	46.0	6.4	14.05	5.2	12.97	4.8	8.12	4	11.12	60.5	20.1
2014-09-11 14:00	51.0	10.9	14.52	6	13.02	6.4	9.52	4.5	10.61	61	21.1
2014-09-11 14:00	51.0	10.9	14.80	6	11.13	6.4	8.23	4.5	14.32	61	21.1
2014-09-12 09:00	70.0	7.5	12.62	4.8	11.07	4.4	7.94	4.4	9.68	62.3	20.6
2014-09-12 09:00	70.0	7.5	13.53	4.8	5.17	4.4	9.85	4.4	9.19	62.3	20.6
2014-09-12 14:00	75.0	7.7	14.57	4.1	12.73	4.9	9.44	5	12.92	41.9	20.8
2014-09-12 14:00	75.0	7.7	14.65	4.1	11.15	4.9	8.69	5	11.42	61.9	20.8
2014-09-13 12:00	97.0	8.8	13.51	4	10.29	4.5	7.49	4.2	12.84	58	20.6
2014-09-13 12:00	97.0	8.8	12.75	4	10.86	4.5	7.32	4.2	13.00	58	20.6
2014-09-14 18:00	127.0	12.7	12.22	4.8	10.65	4	8.79	4.1	12.53	58	20.6
2014-09-14 18:00	127.0	12.7	13.58	4.8	10.54	4	9.19	4.1	11.65	58	20.6
2014-09-15 09:00	142.0	7.9	13.08	4.2	11.39	4.4	7.04	3.7	12.04	58.3	20.3
2014-09-15 09:00	142.0	7.9	13.26	4.2	11.10	4.4	7.42	3.7	10.20	58.3	20.3
2014-09-15 16:30	149.5	9.5	12.19	6.5	10.36	5.1	9.02	6.7	11.98	57.3	20.5
2014-09-15 16:30	149.5	9.5	10.99	6.5	10.01	5.1	7.81	6.7	11.98	57.3	20.5
2014-09-16 09:30	166.5	5.8	10.63	4.1	9.43	4.5	7.29	3.8	9.17	55	20.4
2014-09-16 09:30	166.5	5.8	12.09	4.1	11.32	4.5	8.85	3.8	12.30	55	20.4

Table D-22: Protein and Polysaccharide measurements for Run 6

	Proteins	Abs@562nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)		Polysaccharides	Abs@490nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)
	Zero	0.039						**Zero**	0.02145				
Membrane	**10 ppm, 2 h, daily**	0.168	0.129	113.42	37.81	39.36	**Membrane**	**10 ppm, 2 h, daily**	0.4174	0.39595	28.51	9.50	9.63
		0.1809	0.1419	124.76	41.59				0.4033	0.38185	27.50	9.17	
		0.171	0.132	116.06	38.69				0.4466	0.42515	30.62	10.21	
	100 ppm, 30 min, daily	0.1717	0.1327	116.68	38.89	40.68		**100 ppm, 30 min, daily**	0.3278	0.30635	22.06	7.35	7.96
		0.1703	0.1313	115.44	38.48				0.357	0.33555	24.16	8.05	
		0.1914	0.1524	134.00	44.67				0.3747	0.35325	25.44	8.48	
	10 ppm, 30 min, daily	0.1358	0.0968	85.11	28.37	27.74		**10 ppm, 30 min, daily**	0.2211	0.19965	14.38	4.79	6.08
		0.123	0.084	73.86	24.62				0.2837	0.26225	18.89	6.30	
		0.1421	0.1031	90.65	30.22				0.3195	0.29805	21.46	7.15	
	Control	0.1748	0.1358	119.40	39.80	40.63		**Control**	0.443	0.42155	30.36	10.12	10.27
		0.178	0.139	122.21	40.74				0.5091	0.48765	35.12	11.71	
		0.1801	0.1411	124.06	41.35				0.3961	0.37465	26.98	8.99	
Spacer	**10 ppm, 2 h, daily**	0.101	0.062	54.51	9.09	10.20	**Spacer**	**10 ppm, 2 h, daily**	0.072	0.05055	3.64	0.61	0.37
		0.1162	0.0772	67.88	11.31				0.0321	0.01065	0.77	0.13	
	100 ppm, 30 min, daily	0.0884	0.0494	43.43	7.24	7.74		**100 ppm, 30 min, daily**	0.0931	0.07165	5.16	0.86	0.84
		0.0953	0.0563	49.50	8.25				0.09	0.06855	4.94	0.82	
	10 ppm, 30 min, daily	0.0956	0.0566	49.76	8.29	7.90		**10 ppm, 30 min, daily**	0.0406	0.01915	1.38	0.23	0.44
		0.0902	0.0512	45.02	7.50				0.0764	0.05495	3.96	0.66	
	Control	0.1123	0.0733	64.45	10.74	11.34		**Control**	0.1311	0.10965	7.90	1.32	1.09
		0.1205	0.0815	71.66	11.94				0.0937	0.07225	5.20	0.87	

Table D-23: CFU counts for Run 6

	Dilution	10^2			10^3			10^3				Average count	Average CFU/cm^2
Membrane	10 ppm, 2 h, daily	83	97									90.0	3.00E+04
	100 ppm, 30 min, daily	32	30									31.0	1.03E+04
	10 ppm, 30 min, daily				10	9						9.5	3.17E+04
	Control							109	88	87		94.7	3.16E+06
	Dilution	10^1			10^2		10^3						
Spacer	10 ppm, 2 h, daily	115	101									108.0	1.80E+03
	100 ppm, 30 min, daily	67	40									53.5	8.92E+02
	10 ppm, 30 min, daily	33	50									41.5	6.92E+02
	Control					24	37					30.5	5.08E+02

Table D-24: Cell counts for Run 6

	Dilution	10^1											10^0			
	Membrane												**Spacer**			
	10 ppm.,2 h, daily			**100 ppm, 30 min, daily**			**10 ppm, 30 min, daily**			**Control**			**10 ppm, 2 h, daily**	**100 ppm, 30 min, daily**	**10 ppm, 30 min, daily**	**Control**
	2	3	1	1	3	3	3	1	1	2	1	2	1	1	0	1
	2	0	1	1	2	4	1	1	0	2	1	3	0	1	0	1
	2	3	1	1	0	1	1	0	1	1	0	2	0	1	2	2
	1	4	3	1	4	1	1	1	1	3	4	2	1	2	2	1
	2	2	2	3	1	0	1	1	2	1	3	5	2	0	2	0
	1	1	0	1	5	0	1	2	1	4	1	5	0	0	1	3
	1	1	0	3	1	0	0	4	1	2	4	1	0	1	0	0
	3	1	3	1	2	2	1	1	1	2	0	3	1	1	2	0
	1	2	2	4	3	1	1	1	0	1	1	1	1	0	1	3
	4	0	2	0	2	2	2	2	1	4	2	2	1	2	0	1
	2	0	1	3	2	2	1	1	1	1	3	3	1	1	1	2
	1	3	2	2	0	1	3	1	0	0	1	0	1	1	1	0
	2	2	0	1	1	1	2	4	2	1	3	0	0	0	2	3
	1	3	1	1	2	0	1	2	2	2	3	2	1	1	0	1
	3	2	2	2	1	0	1	1	1	1	1	3	1	1	0	0
	4	2	1	2	2	2	1	3	1	1	2	2	1	1	0	1
	3	2	2	1	1	1	0	1	2	1	4	3	0	0	1	2
	1	1	1	1	2	1	2	1	1	2	2	2	1	2	1	0
	3	2	3	0	3	2	2	3	2	3	1	5	1	1	0	2
	1	3	0	1	3	2	1	3	2	1	1	1	1	0	1	1
Average per square	1.75E+00			1.60E+00			1.38E+00			2.00E+00			7.5E-01	8.5E-01	8.5E-01	1.2E+00
Cell count(cells/µl)	7.00E+05			6.40E+05			5.53E+05			8.00E+05			3.0E+04	3.4E+04	3.4E+04	4.8E+04
Cell count(cells/cm^2)	2.33E+08			2.13E+08			1.84E+08			2.67E+08			1.0E+07	1.1E+07	1.1E+07	1.6E+07

Run 7

Table D-25: Flux, conductivity and temperature measurements for Run 7

		Block 1		Block 2		Block 3		Block 4		Feed	
	Dosing	100 ppm, 30 min, daily		100 ppm, 30 min, every 2nd day		Control		10 ppm, 30 min, daily		100 µg/ℓ C nutrients	
Date	Time (h)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Cond. (µS/cm)	Temp(°C)
2014-10-24 15:00	0.0	7.1	14.47	7.4	13.68	5.1	7.93	5.5	11.26	92	20.7
2014-10-24 15:00	0.0	7.1	14.75	7.4	13.91	5.1	8.25	5.5	11.63	92	20.7
2014-10-24 15:00	0.0	7.1	14.84	7.4	13.94	5.1	8.16	5.5	11.54	92	20.7
2014-10-25 10:00	19.0	7.7	14.52	15.4	14.38	8.9	7.50	7.4	11.60	122.9	22
2014-10-25 10:00	19.0	7.7	14.45	15.4	14.22	8.9	8.45	7.4	11.85	122.9	22
2014-10-26 11:30	44.5	11.8	11.67	18.5	10.82	15.1	6.77	18	9.23	162.7	20.2
2014-10-26 11:30	44.5	11.8	11.05	18.5	10.89	15.1	5.64	18	10.19	162.7	20.2
2014-10-27 09:30	66.5	19.4	10.77	19.8	10.54	10.2	3.73	17.3	8.65	156.9	21.1
2014-10-27 09:30	66.5	19.4	10.80	19.8	9.67	10.2	5.40	17.3	8.68	156.9	21.1
2014-10-27 16:00	73.0	21.9	10.38	33.6	8.31	12.7	5.52	18	9.00	207.8	21.6
2014-10-27 16:00	73.0	21.9	10.23	33.6	10.02	12.7	4.22	18	9.07	207.8	21.6
2014-10-28 09:00	90.0	20.6	8.77	24.9	9.75	11.3	5.53	20.1	8.34	143.7	22.6
2014-10-28 09:00	90.0	20.6	9.29	24.9	9.60	11.3	5.50	20.1	8.48	143.7	22.6
2014-10-28 09:00	90.0	20.6		24.9	10.16	11.3		20.4	8.58	143.7	22.6
2014-10-28 16:00	97.0	16.4	9.15	23.7	8.04	14	5.78	18	7.69	207.6	22.4
2014-10-28 16:00	97.0	16.4	8.40	23.7	7.71	14	4.06	18	7.62	207.6	22.4
2014-10-28 16:00	97.0	16.4	9.27	23.7	8.27	14	5.06	18	7.43	207.6	22.4
2014-10-29 08:30	113.5	22.2	9.43	27.5	7.78	14.9	4.12	16.3	7.75	148.5	22.1
2014-10-29 08:30	113.5	22.2	9.47	27.5	7.53	14.9		16.3	7.07	148.5	22.1
2014-10-29 08:30	113.5	22.2	9.28	27.5	8.39	14.9	4.52	16.3	-29.32	148.5	22.1
2014-10-29 16:00	121.0	17.2	7.75	30.3	8.78	16.5	4.04	16.6	8.23	202.1	22.2
2014-10-29 16:00	121.0	17.2	8.98	30.3	8.55	16.5	4.46	16.6	7.91	202.1	22.2
2014-10-29 16:00	121.0	17.2	8.81	30.3	8.68	11.9	3.57	16.6	-29.32	202.1	22.2
2014-10-30 08:30	137.5	21.8	6.93	17.6	7.05	16.5	6.09	15.5	5.74	124.1	21.6
2014-10-30 08:30	137.5	21.8	7.37	17.6	8.33	11.9	3.81	15.5	6.44	124.1	21.6
2014-10-30 08:30	137.5	21.8	7.28	17.6	8.52	11.9	4.98	15.5	6.00	124.1	21.6
2014-10-30 16:00	145.0	14.1	8.43	26.9	7.23	14.6	5.88	17.2	4.29	192.7	21.1
2014-10-30 16:00	145.0	14.1	6.71	26.9	7.10	14.6	5.28	17.2	6.42	192.7	21.1
2014-10-30 16:00	145.0	14.1	8.22	26.9		14.6	5.71	17.2	6.42	192.7	21.1
2014-10-31 09:30	162.5	18.1	7.74	23.5	6.88	10.5	5.99	15.6	6.75	125.4	20.8
2014-10-31 09:30	162.5	18.1	7.02	23.5	6.72	10.5	5.33	15.6	6.65	125.4	20.8
2014-10-31 09:30	162.5	18.1	7.31	23.5		10.5	4.21	15.6	6.81	125.4	20.8
2014-10-31 15:00	168.0	10.9		23.4	8.35	14.1	3.78	12.9	8.00	195.4	21.9
2014-10-31 15:00	168.0	10.9	9.04	23.4	8.04	14.1	5.91	12.9	7.12	195.4	21.9
2014-10-31 15:00	168.0	10.9	9.05	23.4	8.50	14.1	5.90	12.9	7.86	195.4	21.9
2014-11-01 12:45	189.8	11.7	9.04	26.9	6.00	14.7	3.65	13.7	7.80	195.4	22.2

2014-11-01 12:45	189.8	11.7	8.93	26.9	7.90	14.7	5.56	13.7	7.23	195.4	22.2
2014-11-01 12:45	189.8	11.7	9.21	26.9	8.06	14.7	5.14	13.7	7.72	130.2	22.2
2014-11-02 08:30	209.5	14.9	9.48	26.8	8.13	33.8	4.36	11.6	7.91	130.2	21.7
2014-11-02 08:30	209.5	14.9	9.51	26.8	7.89	33.8	4.39	11.6	8.00	130.2	21.7

Table D-26: Protein and Polysaccharide measurements for Run 7

	Proteins	Abs@562nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)		Polysaccharides	Abs@490nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)
	Zero	0.13205						**Zero**	0.1747				
Membrane	**100 ppm, 30 min, daily**	0.5288	0.5288	464.94	154.98	152.46	**Membrane**	**100 ppm, 30 min daily**	1.7505	1.5758	113.48	37.83	42.08
		0.5088	0.5088	447.36	149.12				2.0527	1.878	135.25	45.08	
		0.523	0.523	459.84	153.28				1.9798	1.8051	130.00	43.33	
	100 ppm, 30 min, every 2nd day	0.6088	0.6088	535.28	178.43	192.49		**100 ppm, 30 min, every 2nd day**	2.4171	2.2424	161.49	53.83	52.53
		0.7104	0.7104	624.61	208.20				2.3371	2.1624	155.73	51.91	
		0.6511	0.6511	572.47	190.82				2.3351	2.1604	155.58	51.86	
	Control	0.6778	0.6778	595.95	198.65	231.00		**Control**	2.9318	2.7571	198.56	66.19	66.14
		0.9818	0.9818	863.24	287.75				2.9168	2.7421	197.48	65.83	
		0.7049	0.7049	619.78	206.59				2.9409	2.7662	199.21	66.40	
	10 ppm, 30 min, daily	0.4298	0.4298	377.90	125.97	129.25		**10 ppm, 30 min, daily**	1.4834	1.3087	94.25	31.42	31.78
		0.4746	0.4746	417.29	139.10				1.4529	1.2782	92.05	30.68	
		0.4186	0.4186	368.05	122.68				1.5594	1.3847	99.72	33.24	
Spacer	**100 ppm, 30 min, daily**	0.2486	0.2486	218.58	36.43	38.96	**Spacer**	**100 ppm, 30 min, daily**	0.643	0.4683	33.73	5.62	5.53
		0.2831	0.2831	248.91	41.49				0.6285	0.4538	32.68	5.45	
	100 ppm, 30 min, every 2nd day	0.2598	0.2598	228.43	38.07	40.75		**100 ppm, 30 min, every 2nd day**	0.2037	0.029	2.09	0.35	2.87
		0.2964	0.2964	260.61	43.43				0.6231	0.4484	32.29	5.38	
	Control	0.6084	0.6084	534.93	89.15	93.07		**Control**	2.432	2.2573	162.56	27.09	26.92
		0.6618	0.6618	581.88	96.98				2.4033	2.2286	160.49	26.75	
	10 ppm, 30 min, daily	0.2512	0.2512	220.87	36.81	36.99		**10 ppm, 30 min, daily**	0.8805	0.7058	50.83	8.47	8.01
		0.2536	0.2536	222.98	37.16				0.8029	0.6282	45.24	7.54	

Table D-27: CFU counts for Run 7

	Dilution	10^3			10^4			10^5				**Average count**	**Average CFU/cm^2**
Membrane	100 ppm, 30 min, daily	116	110	160								128.7	4.29E+05
	100 ppm, 30 min, every 2nd day	108	190	190								162.7	5.42E+05
	Control							135	196			165.5	5.52E+07
	10 ppm, 30 min, daily				139	237	290					274.0	9.13E+05
					430								
	Dilution	10^3		10^4		10^5							
Spacer	100 ppm, 30 min, daily	144	109	25								250.0	4.17E+05
	100 ppm, 30 min, every 2nd day	34	57	90								60.3	1.01E+05
	Control					58						58.0	9.67E+04
	10 ppm, 30 min, daily			37								37.0	6.17E+04

Table D-28: Cell counts for Run 7

Dilution	10^1						10^0			
Membrane							**Spacer**			
100 ppm, 30 min, daily	**100 ppm, 30 min, every 2nd day**	**Control**		**10 ppm, 30 min, daily**		**10 ppm, 2 h, daily**	**100 ppm, 30 min, daily**	**10 ppm, 30 min, daily**	**Control**
7	5	3	8	5	5	3	6	6	2	2	3
4	8	6	8	7	6	3	6	6	3	2	2
7	6	4	5	4	3	3	5	3	4	1	3
4	7	8	6	8	2	1	3	6	3	5	4
3	6	6	5	5	5	3	2	5	4	2	4
4	4	11	5	7	4	2	4	3	4	0	4
4	8	10	6	5	4	2	3	7	4	1	4
7	8	9	4	5	3	4	4	6	5	2	3
6	5	7	7	6	5	4	4	4	5	1	0
5	7	6	7	4		3	5	4	5	0	1
2		7		4		2		2	7	1	3
7		7		5		4		5	6	2	3
4		6		6		3		7	4	0	2
5		2		7		4		6	4	0	3
4		4		4		2		6		1	2
6		6		4		4					
7		7		4		1					
4		9		5		4					
6		6		7		6					
4		5		5		5					
Average per square	5.47E+00		6.33E+00		4.97E+00		3.50E+00	5.1E+00	4.3E+00	1.3E+00	2.7E+00
Cell count(cells/µl)	2.19E+06		2.53E+06		1.99E+06		1.40E+06	2.0E+06	1.7E+06	5.3E+05	1.1E+06
Cell count(cells/cm^2)	7.29E+08		8.44E+08		6.62E+08		4.67E+08	3.4E+08	2.9E+08	8.9E+07	1.8E+08

Run 8

Table D-29: Flux, conductivity and temperature measurements for Run 8

		Block 1		Block 2		Block 3		Block 4		Feed	
	Dosing	100 ppm, 2 h, daily		100 ppm, 2 h, every 2nd day		Control		10 ppm, 2 h, daily		100 µg/ℓ C nutrients	
Date	Time (h)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Cond. (µS/cm)	Temp(°C)
2014-11-04 16:00	0.0	18.7	10.33	18.6	11.95	12.1	14.50	12.3	14.82	86.3	21
2014-11-04 16:00	0.0	18.7	10.40	18.6	12.35	12.1	14.79	12.3	14.91	86.3	21
2014-11-04 16:00	0.0	18.7	10.04	18.6	13.14	12.1	14.69	12.3	15.00	86.3	21
2014-11-05 08:30	16.5	22.3	9.37	28.8	12.51	23.1	12.08	24.4	13.24	86.3	21.6
2014-11-05 08:30	16.5	22.3	9.96	28.8	12.64	23.1	11.37	24.4	13.35	145.5	21.6
2014-11-05 15:00	23.0	22.3	10.76	39.7	13.01	32.8	13.21	44.9	14.65	253.5	23.1
2014-11-05 15:00	23.0	39.5	9.68	39.7	14.22	32.8	12.90	44.9	14.26	253.5	23.1
2014-11-05 15:00	23.0	39.5	9.83	39.7	13.78	32.8	13.71	44.9	13.00	253.5	23.1
2014-11-06 09:00	41.0	39.5	9.91	15.3	13.43	13.9	12.61	24.2	12.96	253.5	23.1
2014-11-06 09:00	41.0	20.7	10.00	15.3	13.47	13.9	12.48	24.2	13.07	154.6	23.1
2014-11-06 16:00	48.0	20.7	10.57	18.4	11.37	14.9	12.13	15	13.17	204.8	21.9
2014-11-06 16:00	48.0	20.1	10.32	18.4	11.96	14.9	11.30	15	13.10	204.8	21.9
2014-11-06 16:00	48.0	20.1	10.08	18.4	12.71	14.9	12.18	15	13.96	204.8	21.9
2014-11-07 08:30	64.5	19.4	9.20	16	10.82	10.9	11.10	11.8	12.28	115.6	22.5
2014-11-07 08:30	64.5	19.4	9.79	16	11.70	10.9	11.09	11.8	12.77	115.6	22.5
2014-11-07 15:00	71.0	16.4	10.69	19.4	12.19	18.3	10.19	19.7	14.25	175.6	22.5
2014-11-07 15:00	71.0	16.2	10.46	19.4	11.83	18.3	10.52	19.7	13.32	175.6	22.5
2014-11-08 11:00	91.0	16.2	9.89	14	11.58	23.1	7.96	20.3	12.40	123.7	22.2
2014-11-08 11:00	91.0	19.6	9.97	14	12.30	23.1	8.16	20.3	12.28	123.7	22.2
2014-11-09 10:00	114.0	19.6	9.22	20.1	10.01	39.6	6.77	24.9	10.63	177.9	21
2014-11-09 10:00	114.0	20.1	8.83	20.1	10.17	39.6	6.76	24.9	10.74	177.9	21
2014-11-10 09:00	137.0	20.1	9.40	19.1	10.42	19.9	6.73	18.3	10.71	177.9	21.6
2014-11-10 09:00	137.0	16.4	9.37	19.1	10.48	19.9	6.86	18.3	10.63	167.3	21.6
2014-11-10 17:30	145.5	16.4	9.89	17.9	10.11	16.7	7.68	14.9	11.70	167.2	22.2
2014-11-10 17:30	145.5	21.5	10.03	17.9	9.99	16.7	7.78	14.9	11.56	196.5	22.2
2014-11-11 07:30	159.5	21.5	9.13	20.1	9.35	19.3	7.07	22	9.61	196.5	21.3
2014-11-11 07:30	159.5	17.6	9.12	20.1	8.89	19.3	6.93	22	10.20	161.6	21.3
2014-11-11 16:00	168.0	17.6	8.99	19.3	9.75	20.9	7.03	19.9	10.53	161.6	22
2014-11-11 16:00	168.0	19	9.49	19.3	9.63	20.9	7.37	19.9	9.56	213.9	22
2014-11-12 08:30	184.5	19	8.95	17.6	8.77	24.4	6.51	18.9	8.78	213.9	21.1
2014-11-12 08:30	184.5	16.5	8.88	17.6	9.88	24.4	6.64	18.9	9.50	160.9	21.1
2014-11-12 16:00	192.0	16.5	8.42	22.7	8.40	18.9	6.84	20.3	9.96	160.9	21.6
2014-11-12 16:00	192.0	18.2	8.94	22.7	8.76	18.9	7.02	20.3	9.97	232	21.6
2014-11-13 08:30	208.5	18.2	8.31	19.7	7.46	17.7	6.44	20.3	8.23	232	20.3
2014-11-13 08:30	208.5	18.2	8.54	19.7	8.08	19.7	6.84	20.3	8.81	162.2	20.3

Table D-30: Protein and Polysaccharide measurements for Run 8

	Proteins	Abs@562nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)		Polysaccharides	Abs@490nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)
	Zero	0.1341						**Zero**	0.04585				
Membrane	**100 ppm, 2 h, daily**	0.418	0.2839	249.62	83.21	82.38	**Membrane**	**100 ppm, 2 h, daily**	1.334	1.28815	92.77	30.92	28.93
		0.4163	0.2822	248.12	82.71				1.381	1.33515	96.15	32.05	
		0.4112	0.2771	243.64	81.21				1.0381	0.99225	71.46	23.82	
	100 ppm, 2 h, every 2nd day	0.5282	0.3941	346.51	115.50	120.27		**100 ppm, 2 h, every 2nd day**	1.9225	1.87665	135.15	45.05	46.01
		0.6269	0.4928	433.29	144.43				1.9102	1.86435	134.26	44.75	
		0.4783	0.3442	302.63	100.88				2.0543	2.00845	144.64	48.21	
	Control	0.8561	0.722	634.81	211.60	231.70		**Control**	2.9241	2.87825	207.28	69.09	69.40
		0.857	0.7229	635.60	211.87				2.876	2.83015	203.82	67.94	
		1.0609	0.9268	814.88	271.63				3.0105	2.96465	213.50	71.17	
	10 ppm, 2 h, daily	0.4339	0.2998	263.60	87.87	85.39		**10 ppm, 2 h, daily**	0.9667	0.92085	66.32	22.11	24.11
		0.4353	0.3012	264.83	88.28				1.0574	1.01155	72.85	24.28	
		0.4072	0.2731	240.12	80.04				1.1262	1.08035	77.80	25.93	
Spacer	**100 ppm, 2 h, daily**	0.1464	0.0123	10.81	1.80	2.12	**Spacer**	**100 ppm, 2 h, daily**	0.136	0.09015	6.49	1.08	1.55
		0.1508	0.0167	14.68	2.45				0.2133	0.16745	12.06	2.01	
	100 ppm, 2 h, every 2nd day	0.1574	0.0233	20.49	3.41	3.36		**100 ppm, 2 h, every 2nd day**	0.0572	0.01135	0.82	0.14	0.21
		0.1567	0.0226	19.87	3.31				0.0701	0.02425	1.75	0.29	
	Control	0.2207	0.0866	76.14	12.69	13.45		**Control**	0.1709	0.12505	9.01	1.50	1.64
		0.2311	0.097	85.29	14.21				0.1945	0.14865	10.71	1.78	
	10 ppm, 2 h, daily	0.1581	0.024	21.10	3.52	3.78		**10 ppm, 2 h daily**	0.1136	0.06775	4.88	0.81	0.76
		0.1617	0.0276	24.27	4.04				0.1042	0.05835	4.20	0.70	

Table D-31: CFU counts for Run 8

	Dilution	10^3			10^4			10^5				**Average count**	**Average CFU/cm^2**
Membrane	100 ppm, 2 h, daily	176	290									233	7.77E+05
	100 ppm, 2 h, every 2nd day	60	39									49.5	1.65E+05
	Control							105	207			156	5.20E+07
	10 ppm, 2 h, daily					90	67					78.5	2.62E+06
	Dilution	10^2		10^3		10^4							
Spacer	100 ppm, 2 h, daily	124	175									149.5	2.49E+04
	100 ppm, 2 h, every 2nd day			37	50							43.5	7.25E+03
	Control					200	197					198.5	3.31E+04
	10 ppm, 2 h, daily			45	60							52.5	8.75E+03

Table D-32: Cell counts for Run 8

	Dilution		10^1										10^1			
	Membrane												**Spacer**			
	100 ppm, 2 h, daily			**100 ppm, 2 h, every 2nd day**			**Control**			**10 ppm, 2 h, daily**			**100 ppm, 2 h, daily**	**100 ppm, 2 h, every 2nd day**	**Control**	**10 ppm, 2 h, daily**
	4	4	4	5	7	3	7	6	6	4	3	3	1	1	2	1
	2	2	5	3	2	4	3	6	2	1	7	5	1	2	4	1
	3	1	4	5	3	5	4	2	6	4	2	1	2	1	1	0
	5	3	3	3	4	2	6	3	5	4	3	1	0	0	2	0
	3	6	5	5	4	6	6	4	4	3	6	3	3	2	7	2
	5	4	0	4	5	3	2	6	3	3	6	2	1	1	8	0
	6	6	7	3	2	2	3	5	5	2	4	3	0	2	3	0
	3	6	3	4	5	6	4	2	3	3	5	3	0	0	4	2
	4	3	4	1	5	6	6	3	3	5	3	4	2	2	4	1
	2	3	7	5	4	2	4	4	4	4	3	7	2	2	3	0
	3	5	4	3	5	3	6	4	3	4	4	3	3	0	4	0
	3	4	8	5	4	6	7	5	4	3	5	4	0	0	3	1
	3	3	2	2	6	4	7	5	3	6	2	3	2	1	4	1
	4	4	4	5	5	3	8	2	5	5	2	3	1	1	5	0
	4	3	5	4	6	3	2	5	4	3	4	4	1	0	3	1
		3											1	0	2	1
													3	1		0
													0	2		1
													0	2		1
													1	3		0
Average per square	3.89E+00			4.04E+00			4.38E+00			3.60E+00			1.2E+00	1.2E+00	3.7E+00	6.5E-01
Cell count(cells/µl)	1.56E+06			1.62E+06			1.75E+06			1.44E+06			4.8E+04	4.6E+04	1.5E+05	2.6E+04
Cell count(cells/cm^2)	5.19E+08			5.39E+08			5.84E+08			4.80E+08			1.6E+07	1.5E+07	4.9E+07	8.7E+06

Run 9

Table D-33: Flux, conductivity and temperature measurements for Run 9

		Block 1		Block 2		Block 3		Block 4		Feed	
	Dosing	10 ppm, 2 h, every 2nd day		10 ppm, 30 min, every 2nd day		Control		10 ppm, 30 min, daily		100 µg/ℓ C nutrients	
Date	Time (h)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Cond. (µS/cm)	Temp(°C)
2014-11-15 13:00	0.0	12.1	14.37	0	14.18	0	14.39	0	15.21	0	19.1
2014-11-15 13:00	0.0	12.1	14.69	0	14.87	0	14.37	0	14.48	0	19.1
2014-11-15 13:00	0.0	12.1	14.76	0	14.97	0	14.48	0	15.36	0	19.1
2014-11-16 14:30	25.5	10.3	17.46	0	17.63	0	17.69	0	18.54	0	23.2
2014-11-16 14:30	25.5	10.3	17.13	0	17.48	0	17.42	0	18.28	0	23.2
2014-11-16 17:00	28.0	10.3	16.03	0	15.12	0	15.58	0	16.00	0	21.2
2014-11-17 06:00	41.0	12.2	13.20	0	13.97	0	13.87	0	15.21	0	21
2014-11-17 06:00	41.0	12.2	14.97	0	14.70	0	15.48	0	14.71	0	21
2014-11-17 16:00	51.0	13	14.64	0	14.94	0	15.10	0	14.53	0	21.1
2014-11-17 16:00	51.0	13	15.11	0	14.86	0	14.29	0	15.59	0	21.1
2014-11-18 08:00	67.0	9.1	14.05	0	13.89	0	14.18	0	13.92	0	21.8
2014-11-18 08:00	67.0	9.1	13.89	0	14.02	0	14.27	0	14.11	0	21.8
2014-11-18 16:00	75.0	8.1	15.02	0	15.32	0	12.66	0	15.61	0	22.7
2014-11-18 16:00	75.0	8.1	15.02	0	15.18	0	13.81	0	15.14	0	22.7
2014-11-18 16:00	75.0	8.1	15.63	0	15.51	0	12.29	0	15.26	0	22.7
2014-11-19 17:30	100.5	6.9	15.21	0	14.83	0	13.18	0	14.86	0	23
2014-11-19 17:30	100.5	6.9	15.13	0	14.41	0	12.23	0	14.61	0	23
2014-11-20 08:00	115.0	10.4	12.10	0	11.03	0	10.27	0	13.38	0	20.1
2014-11-20 08:00	115.0	10.4	11.93	0	11.96	0	10.40	0	13.52	0	20.1
2014-11-20 14:00	121.0	11.8	14.38	0	13.68	0	10.07	0	14.09	0	21.2
2014-11-20 14:00	121.0	11.8	14.15	0	13.96	0	10.19	0	14.08	0	21.2
2014-11-21 09:00	140.0	11.6	12.74	0	12.04	0	9.23	0	15.41	0	22.3
2014-11-21 09:00	140.0	11.6	11.93	0	12.29	0	9.08	0	12.87	0	22.3
2014-11-21 15:00	146.0	20.6	13.05	0	13.18	0	11.42	0	14.18	0	23.4
2014-11-21 15:00	146.0	20.6	13.56	0	13.00	0	10.41	0	14.71	0	23.4
2014-11-22 14:00	169.0	16.6	11.91	0	11.88	0	9.32	0	11.98	0	23.6
2014-11-22 14:00	169.0	16.6	12.02	0	11.90	0	9.38	0	12.73	0	23.6
2014-11-23 11:00	190.0	31	8.34	0	9.84	0	7.59	0	9.59	0	21.4
2014-11-23 11:00	190.0	31	8.83	0	9.88	0	7.68	0	8.97	0	21.4
2014-11-24 09:00	212.0	17.3	9.83	0	8.37	0	5.53	0	9.70	0	22.4
2014-11-24 09:00	212.0	17.3	9.73	0	8.82	0	6.59	0	9.67	0	22.4
2014-11-24 09:00	212.0	17.3	9.93	0	9.30	0	6.48	0	10.67	0	22.4

Table D-34: Protein and Polysaccharide measurements for Run 9

	Proteins	Abs@562nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)		Polysaccharides	Abs@490nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)
	Zero	0.12885						**Zero**	0.06535				
Membrane	**10 ppm, 2 h, every 2nd day**	0.6316	0.50275	442.04	147.35	135.85	**Membrane**	**10 ppm, 2 h, every 2nd day**	1.7626	1.69725	122.23	40.74	37.53
		0.6748	0.54595	480.02	160.01				1.7966	1.73125	124.68	41.56	
		0.4707	0.34185	300.57	100.19				1.3271	1.26175	90.87	30.29	
	10 ppm, 30 min, every 2nd day	0.6403	0.51145	449.69	149.90	144.04		**10 ppm, 30 min, every 2nd day**	1.7437	1.67835	120.87	40.29	41.59
		0.6458	0.51695	454.52	151.51				1.7977	1.73235	124.76	41.59	
		0.5749	0.44605	392.19	130.73				1.8519	1.78655	128.66	42.89	
	Control	1.0188	0.88995	782.48	260.83	266.11		**Control**	2.9303	2.86495	206.32	68.77	68.79
		1.0232	0.89435	786.35	262.12				2.937	2.87165	206.80	68.93	
		1.0685	0.93965	826.18	275.39				2.925	2.85965	205.94	68.65	
	10 ppm, 30 min daily	0.4304	0.30155	265.13	88.38	85.16		**10 ppm, 30 min, daily**	1.1007	1.03535	74.56	24.85	25.30
		0.4302	0.30135	264.96	88.32				1.1308	1.06545	76.73	25.58	
		0.3977	0.26885	236.38	78.79				1.1263	1.06095	76.41	25.47	
Spacer	**10 ppm, 2 h, every 2nd day**	0.1662	0.03735	32.84	5.47	6.17	**Spacer**	**10 ppm, 2 h, every 2nd day**	0.1769	0.11155	8.03	1.34	1.55
		0.1757	0.04685	41.19	6.87				0.2115	0.14615	10.53	1.75	
	10 ppm, 30 min, every 2nd day	0.1905	0.06165	54.21	9.03	9.03		**10 ppm, 30 min, every 2nd day**	0.235	0.16965	12.22	2.04	2.66
		0.1904	0.06155	54.12	9.02				0.3384	0.27305	19.66	3.28	
	Control	0.2071	0.07825	68.80	11.47	11.02		**Control**	0.3759	0.31055	22.36	3.73	3.67
		0.201	0.07215	63.44	10.57				0.3659	0.30055	21.64	3.61	
	10 ppm, 30 min, daily	0.1477	0.01885	16.57	2.76	3.12		**10 ppm, 30 min, daily**	0.0712	0.00585	0.42	0.07	0.66
		0.1526	0.02375	20.88	3.48				0.1689	0.10355	7.46	1.24	

Table D-35: CFU counts for Run 9

	Dilution	10^4			10^5			10^6				Average count	Average CFU/cm^2
Membrane	10 ppm, 2 h, every 2nd day				115	89						102.0	3.40E+07
	10 ppm, 30 min, every 2nd day				159	180						169.5	5.65E+07
	Control							71	37	58		55.3	1.84E+08
	10 ppm, 30 min, daily				32	29						30.5	1.02E+07
	Dilution	10^3		10^4		10^5							
Spacer	10 ppm, 2 h, every 2nd day			54								54.0	9.00E+05
	10 ppm, 30 min, every 2nd day			73	77							75.0	1.25E+06
	Control					24	19					21.5	3.58E+05
	10 ppm, 30 min, daily	100	86									93.0	1.55E+06

Table D-36: Cell counts for Run 9

	Dilution			10^1							10^1					
	Membrane										**Spacer**					
	10 ppm, 2 h, every 2nd day			**10 ppm, 30 min, every 2nd day**			**Control**			**10 ppm, 30 min, daily**			**10 ppm, 2 h, every 2nd day**	**10 ppm, 30 min, every 2nd day**	**Control**	**10 ppm, 30 min, daily**
	4	4	6	8	6	6	6	6	6	6	5	7	5	4	6	0
	3	5	6	7	6	7	6	6	7	5	6	2	2	5	6	2
	4	5	5	8	7	5	7	6	8	6	2	4	0	4	5	1
	5	5	9	7	6	5	6	3	8	7	4	4	1	1	1	3
	4	7	6	6	8	4	8	8	12	2	7	6	2	2	2	3
	7	7	6	8	4	5	4	8	12	3	4	6	2	2	3	1
	8	3	7	9	8	6	8	3	10	5	6	4	1	1	1	3
	3	5	9	7	4	6	4	8	9	5	9	2	4	0	2	3
	7	6	5	6	8	7	8	7	4	6	5	5	3	1	2	1
	3	5	8	8	3	6	3	6	8	8	6	6	1	4	3	3
	6	7	4	6	6	7	6	7	7	4	5	7	1	3	2	3
	8	3	7	6	8	7	8	5	8	5	6	6	3	2	3	0
	2	4	4	6	8	3	8	6	7	4	6	2	1	3	3	3
	8	3	6	7	6	6	6	6	6	5	6	4	1	2	7	1
	6	6	5	7	5	8	5	7	8	4	5	5	1	1	3	2
		3														
Average per square	5.41E+00			6.38E+00			6.78E+00			5.04E+00			1.9E+00	2.3E+00	3.3E+00	1.9E+00
Cell count(cells/µl)	2.17E+06			2.55E+06			2.71E+06			2.02E+06			7.5E+04	9.3E+04	1.3E+05	7.7E+04
Cell count(cells/cm^2)	7.22E+08			8.50E+08			9.04E+08			6.73E+08			2.5E+07	3.1E+07	4.4E+07	2.6E+07

Run 10

Table D-37: Flux, conductivity and temperature measurements for Run 10

		Block 1		Block 2		Block 3		Block 4		Feed	
	Dosing	200 ppm, 30 min, daily		100 ppm, 30 min, 2 x per day		Control		100 ppm, 1 h, daily		100 µg/ℓ C nutrients	
Date	Time (h)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Per Cond. (µS/cm)	Flux (LMH)	Cond. (µS/cm)	Temp(°C)
2014-12-02 11:00	0.0	21.5	15.06	10.7	15.37	8.8	15.40	8.4	16.93	97.2	19
2014-12-02 11:00	0.0	21.5	15.29	10.7	15.53	8.8	15.42	8.4	16.93	97.2	19
2014-12-02 11:00	0.0	21.5	15.29	10.7	15.08	8.8	14.80	8.4	16.43	97.2	19
2014-12-03 09:00	22.0	7.9	16.60	6.4	15.93	8.4	15.36	8.6	17.26	101.4	20.5
2014-12-03 09:00	22.0	7.9	16.58	6.4	16.10	8.4	15.35	8.6	17.26	101.4	20.5
2014-12-03 16:00	29.0	15.4	16.86	15.6	16.12	18.8	15.76	17.7	17.65	166	22.3
2014-12-03 16:00	29.0	15.4	16.90	15.6	16.30	18.8	15.69	17.7	17.83	166	22.3
2014-12-04 09:00	46.0	7.8	16.67	8.6	15.92	8.5	15.68	8.9	16.99	100.7	22.1
2014-12-04 09:00	46.0	7.8	16.73	8.6	16.04	8.5	15.73	8.9	17.14	100.7	22.1
2014-12-04 19:00	56.0	17.4	15.12	14.8	15.04	18.3	13.07	18.7	16.69	182.3	23.8
2014-12-04 19:00	56.0	17.4	15.10	14.8	15.13	18.3	12.88	18.7	16.81	182.3	23.8
2014-12-05 09:30	70.5	8.6	15.32	7.9	15.24	11.7	12.52	11	16.41	112.6	21.2
2014-12-05 09:30	70.5	8.6	14.99	7.9	15.32	11.7	12.36	11	16.46	112.6	21.2
2014-12-05 17:30	78.5	8.1	17.45	21.1	17.08	30.3	15.79	23.1	18.15	154.9	22.8
2014-12-05 17:30	78.5	18.1	17.48	21.1	16.84	30.3	15.94	23.1	17.91	154.9	22.8
2014-12-06 09:00	94.0	16.8	16.13	12.7	15.34	17.8	14.65	17.5	16.39	140.7	21.8
2014-12-06 09:00	94.0	16.8	15.84	12.7	15.21	17.8	14.61	17.5	16.30	140.7	21.8
2014-12-08 09:30	142.5	15	15.08	16.2	14.81	22.2	15.41	25.6	15.58	231.6	21.3
2014-12-08 09:30	142.5	15	15.15	16.2	14.91	22.2	15.32	25.6	15.66	231.6	21.3
2014-12-08 16:00	149.0	10.6	15.27	8.3	15.38	16	14.85	8.9	15.66	140.2	23.5
2014-12-08 16:00	149.0	10.6	15.81	8.3	14.66	16	14.35	8.9	16.01	140.2	23.5
2014-12-09 08:00	165.0	10.3	14.24	10.1	13.56	13.5	11.95	7.3	7.46	100.2	21.4
2014-12-09 08:00	165.0	10.3	14.23	10.1	13.39	13.5	12.00	7.3	13.94	100.2	21.4
2014-12-09 08:00	165.0	10.3	14.16	10.1	13.36	13.5	12.01	7.3	13.98	100.2	21.4
2014-12-09 17:00	174.0	9.1	13.61	7.2	13.89	11.1	12.04	9.6	14.62	139.8	22.1
2014-12-09 17:00	174.0	9.1	13.64	7.2	13.93	11.1	11.44	9.6	13.17	139.8	22.1
2014-12-10 07:30	188.5	6.7	13.19	5.5	13.41	10.8	11.37	6	13.79	104.8	20.5
2014-12-10 07:30	188.5	6.7	13.00	5.5	12.24	10.8	10.76	6	11.59	104.8	20.5
2014-12-10 07:30	188.5	6.7	13.06	5.5	13.48	10.8	10.68	6	14.05	104.8	20.5
2014-12-10 18:00	199.0	8.3	13.88	11.3	12.87	6.1	10.42	13.2	14.08	141.5	22.1
2014-12-10 18:00	199.0	8.3	13.91	11.3	13.61	6.1	10.45	13.2	14.75	141.5	22.1
2014-12-11 08:00	213.0	8.9	12.38	5.4	12.97	8.4	10.10	6.6	13.35	107.1	20.5
2014-12-11 08:00	213.0	8.9	12.26	5.4	13.25	8.4	10.05	6.6	13.43	107.1	20.5
2014-12-11 16:00	221.0	9.8	12.53	10.9	12.86	16.5	9.72	10.1	13.47	166.8	24.2
2014-12-11 16:00	221.0	9.8	12.48	10.9	13.13	16.5	7.66	10.1	13.22	166.8	24.2

2014-12-12 08:00	237.0	8.8	11.63	9	11.04	14.2	7.98	9	12.93	121.1	22.6
2014-12-12 08:00	237.0	8.8	11.18	9	11.04	14.2	8.94	9	11.96	121.1	22.6
2014-12-12 17:30	246.5	14.9	11.10	13.1	10.56	24.4	6.99	14.1	11.65	215.4	23
2014-12-12 17:30	246.5	14.9	10.82	13.1	10.76	24.4	8.01	14.1	12.20	215.4	23
2014-12-13 08:00	261.0	11.5	9.82	10.4	10.21	19.8	7.80	10.2	10.67	150.3	22.9
2014-12-13 08:00	261.0	11.5	9.14	10.4	10.25	19.8	6.75	10.2	11.14	150.3	22.9

Table D-38: Protein and Polysaccharide measurements for Run 10

	Proteins	Abs@562nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)		Polysaccharides	Abs@490nm	Net Abs	Conc. (µg/ml)	Conc. (µg/cm^2)	Average (µg/cm^2)
	Zero	0.13235						**Zero**	0.0525				
Membrane	**200 ppm, 30 min, daily**	0.6689	0.6689	588.12	196.04	198.21	**Membrane**	**200 ppm, 30 min, daily**	2.2656	2.2131	159.38	53.13	52.37
		0.6876	0.6876	604.57	201.52				2.2279	2.1754	156.66	52.22	
		0.6724	0.6724	591.20	197.07				2.2087	2.1562	155.28	51.76	
	100 ppm, 30 min, 2 x per day	0.5962	0.5962	524.20	174.73	175.47		**100 ppm, 30 min, 2 x per day**	1.6145	1.562	112.49	37.50	40.39
		0.6245	0.6245	549.09	183.03				1.7001	1.6476	118.65	39.55	
		0.5754	0.5754	505.91	168.64				1.8904	1.8379	132.36	44.12	
	Control	0.7685	0.7685	675.70	225.23	240.51		**Control**	2.7219	2.6694	192.24	64.08	62.74
		0.8689	0.8689	763.97	254.66				2.3967	2.3442	168.82	56.27	
		0.8245	0.8245	724.93	241.64				2.8795	2.827	203.59	67.86	
	100 ppm, 1 h, daily	0.6336	0.6336	557.09	185.70	184.63		**100 ppm, 1 h, daily**	2.1085	2.056	148.06	49.35	49.62
		0.6078	0.6078	534.40	178.13				2.1127	2.0602	148.37	49.46	
		0.6485	0.6485	570.19	190.06				2.1371	2.0846	150.12	50.04	
Spacer	**200 ppm, 30 min, daily**	0.5587	0.5587	491.23	81.87	80.69	**Spacer**	**200 ppm, 30 min, daily**	1.611	1.5585	112.24	18.71	18.28
		0.5426	0.5426	477.08	79.51				1.5402	1.4877	107.14	17.86	
	100 ppm, 30 min, 2 x per day	0.5376	0.5376	472.68	78.78	76.85		**100 ppm, 30 min, 2 x per day**	1.3961	1.3436	96.76	16.13	15.72
		0.5112	0.5112	449.47	74.91				1.3291	1.2766	91.94	15.32	
	Control	0.6427	0.6427	565.09	94.18	96.94		**Control**	2.716	2.6635	191.81	31.97	32.46
		0.6803	0.6803	598.15	99.69				2.7973	2.7448	197.67	32.94	
	100 ppm, 1 h, daily	0.4673	0.4673	410.87	68.48	66.02		**100 ppm, 1 h, daily**	1.622	1.5695	113.03	18.84	16.41
		0.4337	0.4337	381.33	63.55				1.218	1.1655	83.93	13.99	

Table D-39: CFU counts for Run 10

	Dilution	10^4			10^5				Average count	Average CFU/cm^2
Membrane	200 ppm, 30 min, daily	52	28						40	1.33E+07
	100 ppm, 30 min, 2 x per day	58	47						52.5	1.75E+07
	Control				46	43			44.5	1.48E+08
	100 ppm, 1 h, daily	54	41						47.5	1.58E+07
	Dilution	10^3		10^4						
Spacer	200 ppm, 30 min, daily	22	6						14	2.33E+05
	100 ppm, 30 min, 2 x per day			65	45				55	9.17E+05
	Control			47	65				56	9.33E+05
	100 ppm, 1 h, daily			54	56				55	9.17E+05

Table D-40: Cell counts for Run 10

	Dilution	10^1								10^1						
	Membrane									**Spacer**						
	200 ppm, 30 min, daily			**100 ppm, 30 min, 2 x per day**			**Control**			**100 ppm, 1 h, daily**			**200 ppm, 30 min, daily**	**100 ppm, 30 min, 2 x per day**	**Control**	**100 ppm, 1 h, daily**
	5	3	5	6	5	7	3	3	7	5	7	7	3	0	3	4
	7	6	7	12	8	4	3	2	4	5	7	3	1	1	1	2
	5	10	4	6	7	7	3	2	4	6	6	6	2	4	1	4
	10	7	4	5	8	7	5	5	5	8	7	3	0	3	1	1
	6	9	5	4	3	3	5	4	4	7	9	7	1	4	2	1
	5	6	4	3	4	5	3	5	6	8	7	8	2	0	2	3
	6	7	7	3	5	4	4	3	4	7	5	2	1	2	1	4
	4	7	10	4	6	7	3	9	3	4	5	6	4	1	1	4
	6	7	7	3	5	4	4	5	2	6	6	7	3	2	2	3
	6	9	7	4	6	5	4	6	5	7	7	4	3	2	1	4
	8	7	7	7	9	7	3	5	4	8	5	5	3	5	4	4
	4	9	6	8	4	8	6	3	5	7	9	6	4	4	2	2
	5	7	3	5	7	7	4	7	4	3	7	8	3	5	3	2
	7	6	5	5	7	5	3	5	4	6	6	4	3	3	1	4
	1	7	3	6	5	6	3	3	5	5	5	3	5	3	2	5
	1												7	3	1	2
													3	1	0	2
													5	1	3	3
													3	1	1	2
													5	3	2	2
Average per square	6.02E+00			5.69E+00			4.20E+00			5.98E+00			2.8E+00	2.6E+00	1.8E+00	3.1E+00
Cell count(cells/µl)	2.41E+06			2.28E+06			1.68E+06			2.39E+06			1.1E+05	1.1E+05	7.0E+04	1.2E+05
Cell count(cells/cm^2)	8.03E+08			7.59E+08			5.60E+08			7.97E+08			3.8E+07	3.5E+07	2.3E+07	4.1E+07

End run measurements summary

Table D-41: Summary of biological parameters at end of a run without nutrient dosing

No Additional Nutrients				
Polysaccharide conc. (µg/cm^2)	**Protein conc. (µg/cm^2)**	**Cell count (cells/cm^2)**	**CFU/cm^2**	**Final Flux Fraction**
7.23	34.64	3.91E+08	1.19E+05	0.855
5.20	21.37	4.73E+08	7.67E+04	0.699
4.91	23.53	3.58E+08	2.43E+05	0.648
7.28	37.06	3.96E+08	7.11E+04	0.808
9.28	29.32	3.20E+08	2.93E+05	0.925
7.62	32.33	2.71E+08	1.88E+05	0.661
4.61	15.20	2.24E+08	1.46E+05	0.966
10.53	24.32	3.38E+08	7.48E+06	0.867
7.99	49.18	4.33E+08	2.56E+05	0.844
13.47	50.07	2.89E+08	9.33E+04	0.787
8.49	29.95	2.71E+08	7.67E+04	0.838
16.44	36.78	3.58E+08	4.58E+06	0.837
8.03	23.77	1.62E+08	4.67E+03	0.748
12.10	36.99	2.58E+08	4.33E+04	0.756
8.47	19.97	1.78E+08	2.22E+04	0.788
9.63	39.36	2.33E+08	3.00E+04	0.712
7.96	40.68	2.13E+08	1.03E+04	0.650
6.08	27.74	1.84E+08	3.17E+04	0.934
10.27	40.63	2.67E+08	3.16E+06	0.736

Table D-42: Summary of biological parameters at end of a run with nutrient dosing

Nutrients: 100 µg/ℓ C Added to Feed water				
Polysaccharide conc. (µg/cm^2)	**Protein conc. (µg/cm^2)**	**Cell count (cells/cm^2)**	**CFU/cm^2**	**Final Flux Fraction**
42.08	113.76	7.29E+08	4.29E+05	0.630
52.53	153.78	8.44E+08	5.42E+05	0.563
66.14	192.29	6.62E+08	5.52E+07	0.524
31.78	90.55	4.67E+08	9.13E+05	0.671
28.93	82.38	5.19E+08	7.77E+05	0.851
46.01	120.27	5.39E+08	1.65E+05	0.645
69.40	231.70	5.84E+08	5.20E+07	0.469
24.11	85.39	4.80E+08	2.62E+06	0.592
37.53	135.85	7.29E+08	4.03E+07	0.615
41.59	144.04	8.50E+08	5.65E+07	0.563
68.79	266.11	9.04E+08	1.84E+08	0.413
25.30	85.16	6.73E+08	1.02E+07	0.618
52.37	159.42	8.03E+08	1.33E+07	0.559
40.39	136.68	7.59E+08	1.75E+07	0.598
62.74	201.72	5.60E+08	1.48E+08	0.428
49.62	145.84	7.97E+08	1.58E+07	0.582